

Insights of hepatozoonosis transmitted by *H. Canis*

Percepção da hepatozoonose transmitida por H. Canis

Anna Luisa Pizzaia Henrique *(ORCID 0009-0002-2010-685X), **Lívia Mendes Miranda** (ORCID 0000-0003-0071-2633),
Leonardo Castilioni (ORCID 0009-0002-3760-269X)

Anhembi Morumbi University, São Paulo, SP, Brazil. *Author for correspondence: anpizzaia@gmail.com

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ABSTRACT

Hepatozoonosis caused by *H. Canis* is a hemoparasitic disease transmitted by the tick *Rhipicephalus sanguineus*. In vertebrate hosts, the disease primarily occurs through ingestion of the hemoparasite or its components. Hepatozoon spp. infection typically remains subclinical, only becoming diagnosed upon microscopic observation of the hemoparasite during blood smear analysis. However, in certain instances, moderate or severe clinical manifestations may occur. Due to limited research focusing on Hepatozoonosis, diagnosis can present challenges, as well as selecting the appropriate test to confirm it, with PCR being considered the preferred method for detection. A two-and-a-half-month-old Golden Retriever puppy was examined, exhibiting symptoms of vomiting, lethargy, bloody diarrhea, anorexia, and hyperthermia (39°C - 102,2°F). Diagnosis was confirmed by observation of Hepatozoon spp. parasites in the blood smear, alongside severe anemia, presence of microagglutination, and elevated total protein levels. The treatment was initiated with Doxycycline, and following the patient's improvement, the owner opted to proceed with castration and subsequently administer two doses of Imidocarb Dipropionate at 5mg/kg with a 14-day interval.

KEYWORDS: Hepatozoon spp.; Hepatozoonosis; small animals; *Rhipicephalus sanguineus*; hemoparasitosis

RESUMO

Hepatozoonose causada por *H. Canis* é uma doença hemoparasitária transmitida pelo carrapato *Rhipicephalus sanguineus*. Nos hospedeiros vertebrados, a doença ocorre principalmente através da ingestão do hemoparasita ou de seus componentes. A infecção por *Hepatozoon spp.* geralmente permanece subclínica, sendo diagnosticada apenas mediante observação microscópica do hemoparasita durante a análise de esfregaço sanguíneo. No entanto, em certas ocasiões, podem ocorrer manifestações clínicas moderadas ou graves. Devido à pesquisa limitada focada na Hepatozoonose, o diagnóstico pode apresentar desafios, assim como a seleção do teste apropriado para confirmá-lo, sendo que a PCR é considerada o método preferido para detecção. Um filhote de Golden Retriever com dois meses e meio de idade foi examinado, apresentando sintomas de vômito, letargia, diarreia sanguinolenta, anorexia e hipertermia (39°C - 102,2°F). O diagnóstico foi confirmado pela observação de parasitas *Hepatozoon spp.* no esfregaço sanguíneo, juntamente com anemia severa, presença de microaglutinação e níveis elevados de proteína total. O tratamento foi iniciado com Doxiciclina, e após a melhora do paciente, o proprietário optou por prosseguir com a castração do animal e subsequentemente administrar duas doses de Imidocarb a 5mg/kg com um intervalo de 14 dias.

PALAVRAS-CHAVE: *Hepatozoon spp.*; Hepatozoonose; pequenos animais; *Rhipicephalus sanguineus*; hemoparasitose.

INTRODUCTION

Hepatozoon spp. is a protozoan responsible for causing Hepatozoonosis, a hemoparasitic disease transmitted by arthropods, with the main biological vector being the tick *Rhipicephalus sanguineus* (O'DWYER et al. 2001, BANETH 2011). Among cases of canine infected with *Hepatozoon spp.*, two species of the protozoan have been identified: *Hepatozoon canis* and *Hepatozoon americanum*, with *H. canis* being the most common in tropical, subtropical, and temperate regions (DÍAZ-SÁNCHEZ et al. 2021). In Brazil, the infection presents low pathogenicity and is frequently associated with other hemoparasites, being more common in countryside areas of Rio de Janeiro, São Paulo, Rio Grande do Sul, Espírito Santo and Minas Gerais (Figure 1) (AGUIAR et al. 2004, MASSARD 1979, RUBINI et al. 2008, FORLANO et al. 2005). According to DÍAZ-SÁNCHEZ et al. (2021), stray dogs or shelter dogs are more susceptible to infection compared to pet dogs, as the prevalence of *H. canis* may vary depending on the population density of the vector (DÍAZ-SÁNCHEZ et al. 2021).

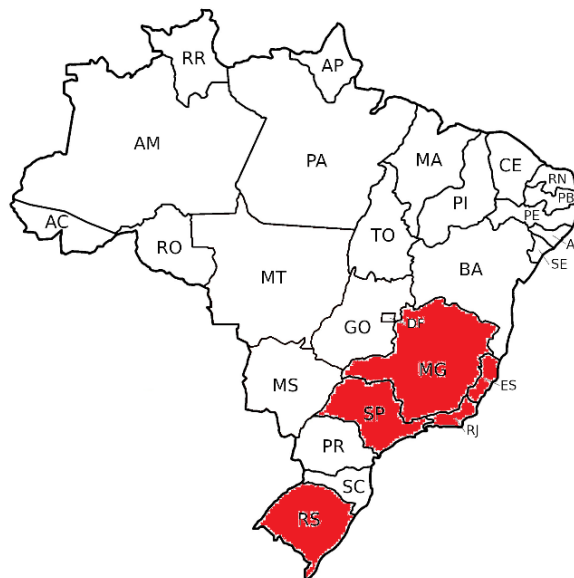


Figure 1. Map of Brazilian Endemic Areas. Source: personal collection.

The life cycle of all species of *Hepatozoon spp.* is divided into two parts that occur in different hosts. When referring to hematophagous invertebrate hosts, such as ticks, the cycle consists of gamogony and sporogony. Regarding vertebrate hosts, there is merogony and gametogony (SCHÄFER et al. 2022).

In the tick life cycle, the transmission of *Hepatozoon spp.* occurs during its larva-to-nymph phase, when it initiates feeding on the blood of vertebrate animals (SCHÄFER et al. 2022). Therefore, for the tick to become infected with the protozoan, it merely needs to feed on the blood of an infected host containing gamonts, cells that divide into same-sex gametes, of *H. canis* that are present within circulating defense cells, such as neutrophils and monocytes (DEMONER et al. 2013). Subsequently, gametogenesis commences at the threshold of the tick's intestine, followed by sporogony in its hemocele, resulting in the production of sporozoites, which are infective to the host. Upon ingestion of these sporozoites by the animal, the cycle persists (CHRISTOPHERS 1912, BANETH et al. 2007, SCHÄFER et al. 2022).

The primary transmission route of the disease to vertebrate hosts occurs through direct ingestion or ingestion of tick parts containing mature oocysts with sporozoites, affecting mammals, reptiles, amphibians, and birds, thereby rendering them intermediate hosts, while fleas, ticks, and insects serve as definitive hosts (DÍAZ-SÁNCHEZ et al. 2021, BANETH 2003, RUBINI et al. 2005, PEREIRA 2007). Nonetheless, instances of transplacental transmission have been documented, whereby the infected mother transmits the protozoan to her offspring (DÍAZ-SÁNCHEZ et al. 2021). A study conducted by SCHÄFER et al. (2022) involving a mother and eight canine offspring reported that one of the puppies was stillborn, with positive *H. canis* DNA detected in the umbilical cord, spleen, and amniotic fluid, while parasite PCR tests on the liver and bone marrow yielded negative results.

When the dog ingests the tick containing oocysts with sporozoites, the sporozoites are released into its gastrointestinal tract, where they penetrate the intestinal wall and enter the bloodstream and lymphatic

system. Subsequently, merogony (asexual reproduction of the protozoan) commences in the lymphoid tissues, potentially affecting the bone marrow (SCHÄFER et al. 2022). By the 13th day post-infection, the merozoites infiltrate neutrophils and monocytes to develop into gamonts, the infectious stage for the tick (SCHÄFER et al. 2022, BANETH et al. 2007).

The level of parasitemia in the animal's body, the presence of other infections caused by different vectors, and even immunosuppressive chemotherapy are what define the degree of symptomatology that the dog will have. Therefore, we can have patients with severe symptoms or, as more frequently observed in clinical practice, subclinical patients (SCHÄFER et al. 2022, BANETH 2003).

In clinical practice, mild normochromic anemia is recognized as one of the indicators of *H. canis* infection (SCHÄFER et al. 2022). However, a comparative analysis of hematological parameters in infected animals conducted by DÍAZ-SÁNCHEZ et al. (2021), revealed no significant alterations aside from anemia, which included leukopenia, thrombocytopenia, and neutropenia. Additionally, a study by SCHÄFER et al. (2022) involving dogs under 18 months of age infected with *H. canis* identified lymphocytosis, monocytosis, and eosinophilia.

Studies indicate that in canine puppies, infections caused by *H. canis* may result in systemic illnesses characterized by clinical manifestations including lethargy, fever, anorexia, pale mucous membranes, weight loss, and gastrointestinal symptoms, being the most prevalent clinical presentations (SCHÄFER et al. 2022, KAUR et al. 2020).

For the diagnosis and detection of *Hepatozoon spp.*, several diagnostic methods are available, including microscopic examination of peripheral blood smears and leukocyte coating analysis, which identifies gamonts in the cytoplasm of neutrophils and occasionally in the cytoplasm of monocytes (DÍAZ-SÁNCHEZ et al. 2021). It is noteworthy that gamonts are accidental findings in blood smears and may appear in canine blood 28 days post-infection. Therefore, microscopic analysis may not be optimal, particularly for patients with low parasitemia, where the circulating gamont count is low (SCHÄFER et al. 2022, DÍAZ-SÁNCHEZ et al. 2021). Given this, PCR is the standard diagnostic test for Hepatozoonosis, as it offers higher sensitivity and specificity compared to blood smears, enabling the detection of *H. canis* in dogs with low parasitemia levels (SCHÄFER et al. 2022, DÍAZ-SÁNCHEZ et al. 2021).

There is currently no officially approved medication for the treatment of *H. canis* infection, posing challenges to therapeutic interventions (SCHÄFER et al. 2022). Nevertheless, in the study conducted by Schafer et al., Imidocarb Dipropionate was administered. However, research indicates that this medication, when administered at its recommended dosage, does not eradicate the disease in animals but rather diminishes parasitemia levels, a process that may occur gradually and necessitate multiple treatments with Imidocarb Dipropionate, eventually leading to the resolution of clinical symptoms. In cases where dogs exhibit low parasitemia, the prognosis for the disease is favorable (SCHÄFER et al. 2022).

CLINICAL CASE

On January 17, 2023, a two-and-a-half-month-old Golden Retriever puppy presented with vomiting, lethargy, bloody diarrhea, anorexia, and hyperthermia (39.9 °C – 102,2 °F) at a clinic in São Paulo. During the anamnesis, the owner reported purchasing the dog from a kennel in the interior of São Paulo, where deworming had been performed. It was also mentioned that episodes of bloody diarrhea began on the second day of living with the animal, starting with soft stools and progressing to more liquid stools, along with coprophagia (Figure 2). Two days after the onset of diarrhea, vomiting started, along with lethargy and anorexia. The patient only drank water when offered while lying down. A fecal parasitological examination was conducted, yielded a negative result, and the consistency of the stools had a mucoid appearance.



Figure 2. Patient's stool at the beginning of the condition.

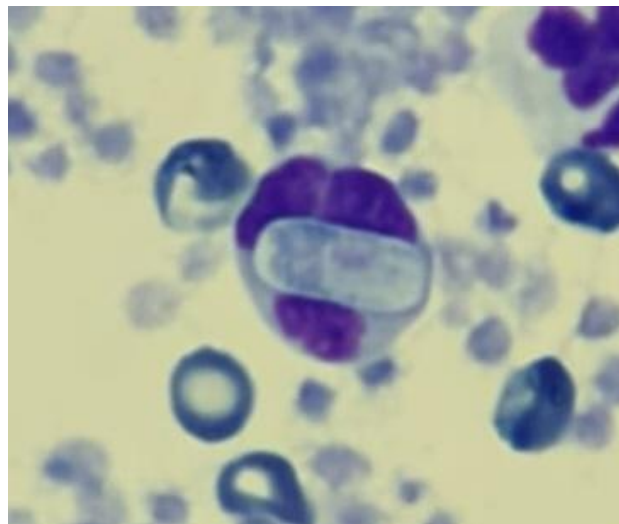


Figure 3. Presence of the *Hepatozoon spp.* parasite in leukocyte.

On January 18, 2023, with no improvement in the patient's condition, the patient returned to the clinic, where subcutaneous fluid therapy with 250 ml of Ringer Lactate was administered, along with Buscopan and Omeprazole. The owner reported that the patient showed no interest in the offered dry food, so Hill's Urgent Care wet food was offered and consumed by the puppy in the clinic. Samples were collected for Parvovirus PCR and a complete blood count. During the blood collection from the cephalic vein, a tick was found on the animal. When asked, the owner denied having other animals at home or the puppy having any contact with grass.

The PCR result for Parvovirus returned negative, while the complete blood count revealed severe anemia, elevated total protein levels and marked microagglutination. Additionally, leukocytosis accompanied by an increase in segmented neutrophils, lymphopenia, and the presence of *Hepatozoon spp.* inclusions were noted (Figure 3). A slight presence of hyposegmented neutrophils was also observed (Table 1 and Table 2). Based on these findings, Doxycycline was administered at a dosage of 10 mg/kg to the patient every 24 hours for a duration of 28 days, instead of Imidocarb Dipropionate, taking into consideration the patient's age and findings from a study conducted by SANTOS et. al. (2019). The study suggests that the sole use of Imidocarb Dipropionate did not yield satisfactory outcomes; however, when combined with antibiotics such as Doxycycline, it demonstrated improved efficacy.

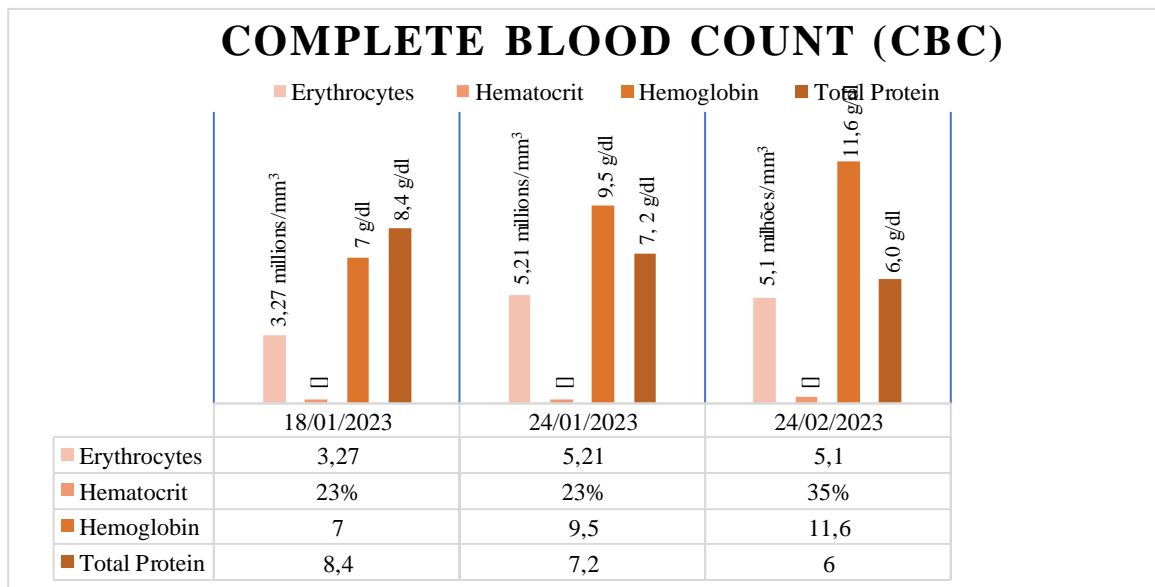


Table 1. Comparison of red blood cell count during the disease discovery period and treatment period.

During the patient’s treatment, the owner noted an improvement in diarrhea two days after the initiation of Doxycycline administration. However, even after six days of treatment, the stools maintained a soft consistency. At a follow-up consultation on January 24, 2023, the owner reported a return to normal appetite and observed no signs of lethargy in the animal. A subsequent complete blood count was conducted to monitor the progression of the infection. Despite ongoing treatment, the patient remained anemic, but the levels of red blood cells, total protein, and hemoglobin showing increases, albeit remaining below the reference range. Furthermore, mild anisocytosis and evidence of both macro and microagglutination were observed. A minor leukocytosis persisted in comparison to the previous examination, primarily attributable to eosinophilia. Platelet evaluation revealed a discreet presence of platelet aggregates, along with a slight detection of *Hepatozoon spp.* within macrophages.

Due to the patient’s improvement, in May 2023, the owner chose to have the dog neutered so that it could stay at daycare while she worked. Pre-surgical exams were conducted on the animal, and all serum levels were within normal range. Additionally, weight gain was observed. One week after the surgical procedure, when the patient was seven months old, two doses of Imidocarb Dipropionate 5 mg/kg were administered with a 14-day interval. Following the treatment, the patient exhibited complete resolution of the condition.

CONCLUSION

In general, *Hepatozoon spp.* infection typically presents as subclinical, often only considered as a potential diagnosis when found in the blood smears. As an obligatory intracellular parasite, it relies on the host cell for survival, particularly within mammalian erythrocytes, primarily affecting domestic carnivores, leading to anemia, and compromising hematological function. However, the disease can also manifest in a moderate or severe manner, causing clinical symptoms such as anorexia, fever, and gastrointestinal issues, as observed in the case described in this study, and may even result in death. It is essential to have a comprehensive understanding of this hemoparasitosis and to conduct further research to aid in more accurate clinical diagnosis, particularly when dealing with the subclinical and asymptomatic presentation of the disease. Additionally, it is crucial to comprehend the biological vector the disease, namely the tick *Rhipicephalus sanguineus*, as it is responsible not only for transmitting this disease but also other zoonotic pathogens.

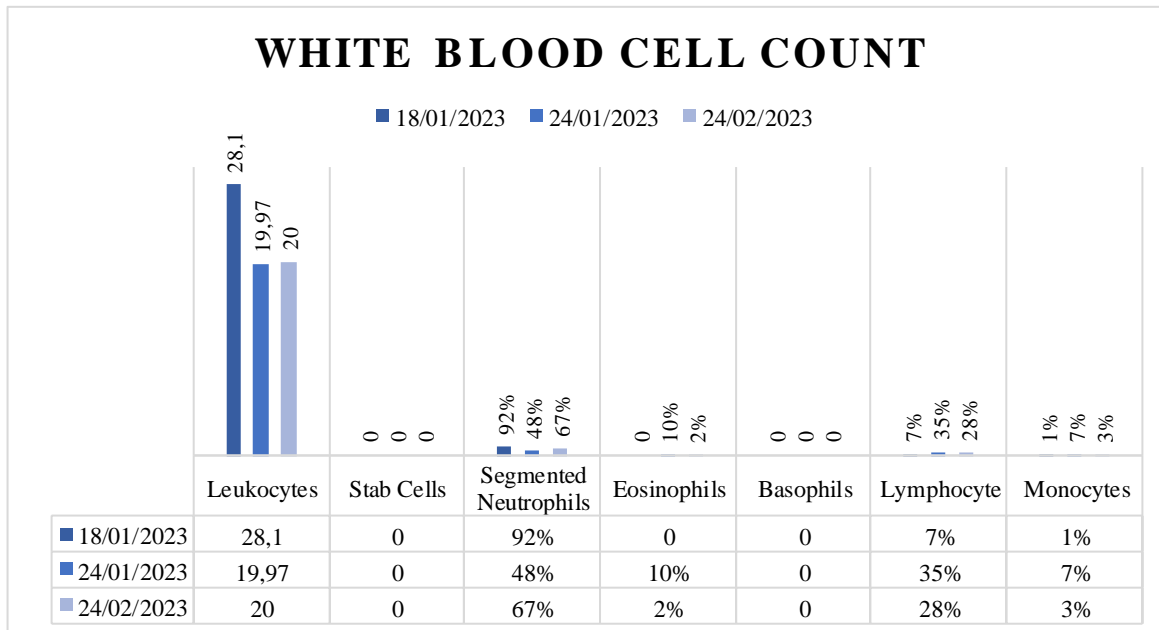


Table 2. Comparison of white blood cell count during the disease discovery period and treatment period.

REFERENCES

AGUIAR DM et al. 2004. Hepatozoonose canina: achados clínico epidemiológicos em três casos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 56: 411-413.

BANETH G. 2003. Disease risks for the travelling pet: Hepatozoonosis. In Practice 25: 272-277.

BANETH G. 2011. Perspectives on canine and feline hepatozoonosis. Veterinary Parasitology 181: 3 – 11.

BANETH G et al. 2007. Life cycle of Hepatozoon canis (Apicomplexa: adeleorina: Hepatozoidae) in the tick Rhipicephalus sanguineus and domestic dog (Canis familiaris). J Parasitol. 93: 283-99.

CHRISTOPHERS SR. 1912. The development of Leucocytozoon canis in the tick with a reference to the development of Piroplasma. Parasitology 5: 37-48.

DEMONER LC et al. 2013. Hepatozoonose canina no Brasil: aspectos da biologia e transmissão. Vet. e Zootec 20: 193-202.

DÍAZ-SÁNCHEZ AA. 2021. Molecular detection and characterization of Hepatozoon canis in stray dogs from Cuba. Parasitol Int. 80: 102200.

FORLANO M et al. 2005. Diagnosis of Hepatozoon spp. in Amblyomma ovale and its experimental transmission in domestic dogs in Brazil. Veterinary Parasitology 134: 1-7.

KAUR N et al. 2020. Development and application of multiplex PCR assay for the simultaneous detection of Babesia vogeli, Ehrlichia canis and Hepatozoon canis in dogs. Acta Trop. 212: 105713.

MASSARD CA. 1979. Hepatozoon canis (James, 1905) (Adeleida: Hepatozoidae) cães do Brasil, com uma revisão do gênero em membros da ordem carnívora. Dissertação (Mestrado em Ciências Veterinárias). Seropédica: UFRRJ.

O'DWYER LH et al. 2001. Hepatozoon canis infection associated with dog thicks of rural areas of Rio de Janeiro state, Brazil. Veterinary Parasitology. 94:143-50.

PEREIRA AM. 2007. Hepatozoonose canina: aspectos gerais da infecção por Hepatozoon Canis em cães (Canis familiaris) no Brasil e no mundo. Monografia (Especialização). Realengo: UCB.

RUBINI AS et al. 2008. Molecular and parasitological survey of Hepatozoon canis (Apicomplexa: Hepatozoidae) in dogs from rural area of São Paulo state, Brazil. Parasitol Res 102: 895-899.

RUBINI AS et al. 2005. Molecular identification and characterization of canine Hepatozoon species from Brazil. Parasitology Research. 97: 91-93.

SANTOS CM et al. 2019. Hepatozoonose Canina: Relato de Caso. Uniciências 23: 12 -15.

SCHÄFER I et al. 2022. First evidence of vertical Hepatozoon canis transmission in dogs in Europe. Parasites Vectors. 23: 296.