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Case report: *Hepatozoon* sp. in a canine in Bogotá

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ABSTRACT

This case report describes a diagnosis of hepatozoonosis in Bogotá in a one-year-old mixed-breed female dog, successfully treated with imidocarb dipropionate. The patient initially presented with persistent cough, organomegaly, poor body condition, and mucosal pallor. A complete blood count revealed normocytic normochromic anemia, thrombocytopenia, and hyperglobulinemia, suggesting hemoparasitism as the primary differential diagnosis. *Hepatozoon* sp. was confirmed by Polymerase Chain Reaction (PCR), leading to the initiation of specific treatment with imidocarb dipropionate, supplemented with atropine and doxycycline to target potential coinfections. Clinical and hematological follow-up showed significant improvement, with resolution of anemia and thrombocytopenia. This case highlights the importance of including *Hepatozoon canis* in the differential diagnoses in areas above 2,600 meters above sea level, particularly in dogs from shelters, due to the risk of underdiagnosis in non-endemic areas and the potential for vertical transmission. The occurrence of hepatozoonosis in Bogotá suggests an emerging risk for the region, emphasizing the need to disseminate this case along with the diagnostic and therapeutic approaches used. In this context, the availability of molecular tools, such as PCR, is crucial for accurate diagnosis and effective management of this infection in newly affected areas.

Keywords: hemoparasites, imidocarb, Colombia, emerging risk.

Reporte de caso: *Hepatozoon* sp. en un canino en Bogotá

RESUMEN

Este reporte de caso describe un diagnóstico de hepatozoonosis en Bogotá en una hembra canina mestiza de un año tratada exitosamente con dipropionato de imidocarb. La paciente presentó inicialmente tos persistente, organomegalia, condición corporal baja y palidez de las mucosas. Tras realizar un hemograma, se detectó anemia normocítica normocrómica, trombocitopenia e hiperglobulinemia, lo que sugirió hemoparasitismo como primer diagnóstico diferencial. La confirmación de *Hepatozoon* sp. mediante PCR permitió iniciar un tratamiento específico con dipropionato de imidocarb, complementado con atropina y doxiciclina dirigida contra posibles coinfecciones. El seguimiento clínico y hematológico mostró una notable mejoría, con resolución de la anemia y la trombocitopenia. Este caso resalta la importancia de incluir *Hepatozoon canis* en los diagnósticos diferenciales en zonas de altitud superior a los 2.600 m s. n. m., particularmente en caninos provenientes de refugios, debido al riesgo de subdiagnóstico en áreas no endémicas y la posibilidad

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de transmisión vertical. La aparición de hepatozoonosis en Bogotá sugiere un riesgo emergente para la región, lo que subraya la necesidad de divulgar este caso junto con los métodos diagnósticos y terapéuticos empleados. En este contexto, la disponibilidad de herramientas moleculares, como la PCR, es crucial para un diagnóstico preciso y un manejo adecuado de esta infección en zonas de reciente incidencia.

Palabras clave: hemoparásitos, imidocarb, Colombia, riesgo emergente.

INTRODUCTION

Canine hepatozoonosis is a parasitic disease caused by protozoa of the genus *Hepatozoon*, belonging to the phylum Apicomplexa, class Conoidasida, and family Hepatozoidae. These parasites have complex biological cycles that involve both vertebrate and invertebrate hosts, with hematophagous ticks acting as vectors. In dogs, the two species of greatest clinical significance are *Hepatozoon canis* and *Hepatozoon americanum*. While *H. canis* typically causes mild to moderate pathogenicity, *H. americanum* is associated with severe clinical manifestations, such as myositis and myocarditis, which can significantly compromise the health of infected dogs (Mastrantonio *et al.* 2023). The primary route of transmission of these protozoa in dogs is through the ingestion of infected ticks (Ewing & Panciera 2003).

Regarding its epidemiology, *Hepatozoon canis* utilizes the tick *Rhipicephalus sanguineus*, commonly known as the brown dog tick, as its definitive host (Freire 2019). Although there are reports identifying *Hepatozoon americanum* oocysts in *Amblyomma maculatum* ticks, these are not considered significant in the transmission of the parasite (Arcila *et al.* 2005). In Brazil, A*mblyomma cajennense* has been determined to lack importance in the transmission of *Hepatozoon canis*, suggesting that this tick does not play a relevant role in the epidemiology of canine hepatozoonosis in the region. Nevertheless, the potential existence of different subspecies of *Amblyomma ovale* with varying capacities to act as pathogen vectors has been proposed, highlighting the need for further studies to clarify the impact of these subspecies on the transmission of *Hepatozoon canis* and other infectious agents (Demoner *et al.* 2013). Additionally, while Rubini *et al.* (2009) suggested that *Amblyomma ovale* might be implicated in the transmission of *Hepatozoon canis,* a significant impact of this species on the epidemiology of the disease has yet to be confirmed. Therefore, further studies are required to more accurately assess its role as a vector and its relevance in the transmission cycle of this parasite.

The primary route of transmission for *Hepatozoon canis* is through the ingestion of infected ticks. The parasite's life cycle begins when the tick ingests gamonts while feeding on the blood of an infected dog, leading to the development of oocysts within the vector. Once the dog ingests the tick carrying the oocysts, these pass through the gastrointestinal wall (Beugnet *et al.* 2018), releasing sporozoites. The sporozoites invade mononuclear phagocytic cells and endothelial cells of various organs, such as the bone marrow, spleen, muscles, liver, and lungs. In these tissues, cysts containing different forms of meronts are formed, which perpetuate the infection within the host (Ettinger *et al.* 2017).

According to the study by Schäfer *et al.* (2022), vertical transmission of *Hepatozoon canis* in dogs has also been identified. Additionally, Beneth (2011) reported that this hemoparasite can be transmitted through the consumption of infected prey. In most cases, *Hepatozoon canis* infection is asymptomatic, and it is common to suspect the infection when a positive patient is identified without evident clinical signs. However, clinical signs can range from mild to severe, depending on the level of parasitemia, the host's immune status, and the presence of coinfections. Dogs with low parasitemia are usually asymptomatic, while those with high parasitemia may exhibit symptoms such as lethargy, fever, and emaciation (Tuna *et al.* 2021). In contrast, *Hepatozoon americanum* infection is associated with more severe clinical signs, including painful myositis, fever, progressive muscle atrophy, weakness, and lameness, which in some cases can lead to paralysis (Eiras *et al.* 2007).

In various regions of the world, coinfections of *Hepatozoon canis* with other pathogens have been described, including *Babesia* sp. (Ciuca *et al.* 2021), *Anaplasma platys* (Anderson *et al.* 2013), and *Ehrlichia canis* (Sukara *et al.* 2023), as well as with distemper, parvovirus, *Anaplasma phagocytophilum, Toxoplasma gondii*, and *Leishmania infantum* (Baneth 2011). It is essential to carefully interpret clinical signs, differentiating those caused by *Hepatozoon canis* from symptoms attributable to other concomitant pathogens. This distinction is particularly important in dogs from shelters or regions with poor sanitary and ectoparasite control, to ensure proper diagnosis and treatment of all present conditions (Greene 2012).

Regarding diagnosis, a study conducted in Argentina by Vezzani (2017) found that 56.9% of dogs infected with *Hepatozoon canis* presented with anemia, 36.3% showed leukocytosis, and 7.5% had leukopenia. Among parasitemic dogs, 74.1% exhibited an inflammatory leukogram, 46.4% had eosinophilia, and 17.8% had monocytosis. Hyperglobulinemia (Beugnet *et al.* 2018) and anemia are associated with the chronicity of the infection (Paiz *et al.* 2016). Thrombocytopenia is uncommon, except in cases of coinfection with other hemoparasites (Ettinger *et al.* 2017). According to Tuna *et al.* (2021), 84.38% of patients coinfected with *Ehrlichia canis* presented with thrombocytopenia, while 56.25% showed anemia, being these the most common hematological abnormalities.

The definitive diagnosis of hepatozoonosis is made through blood smear analysis, where gamonts can be visualized in the cytoplasm of neutrophils and occasionally within monocytes (Mastrantonio *et al.* 2023). However, both blood smears and buffy coat preparations have low sensitivity in animals with subclinical infections due to variable parasitic loads, with a positivity rate not exceeding 5% in infected patients (Modrý *et al.* 2017; Arcila *et al.* 2005). Therefore, PCR is considered the best diagnostic option, as it has been shown to be 2.5 times more sensitive (Karagenc *et al.* 2006).

The most commonly used treatment protocol for *Hepatozoon canis* includes the administration of two doses of imidocarb dipropionate at concentrations of 5 to 6 mg/kg, with a 14-day interval between doses (Plumb 2018). This treatment is frequently supplemented with doxycycline, administered orally at a dose of 10 mg/kg per day for 21 days, to address potential coinfections with rickettsiae (Greene 2012). With less success, toltrazuril therapy has also been used at concentrations of 10-14 mg/kg orally, every 24 hours for 7-10 consecutive days (Beugnet *et al.* 2018). Treatment follow-up should include hematological assessments 14 or 28 days after initiation (Mastrantonio *et al.* 2023). However, prevention remains key, minimizing tick exposure and administering acaricidal products in cases of high-risk exposure (Beugnet *et al.* 2018).

The first report of this hemoparasite in Colombia was in 2005, following the observation of inclusion bodies in neutrophils and monocytes in blood smears (Arcila *et al.* 2005). Isolated cases in Colombia have been of the same class as those from Venezuela, Spain, and Taiwan. The identification of *Hepatozoon canis* in Colombia was accomplished using PCR, and it was first diagnosed in a study conducted with dogs from shelters in Bogotá, Bucaramanga, and Villavicencio, recording a prevalence of 31.8%. However, in that study, no positive animals were found in Bogotá (Vargas–Hernández *et al.* 2011), so the prevalence of hepatozoonosis in the city remains unknown.

Since the initial discovery of *Hepatozoon canis* in Colombia, several studies have reported its prevalence in various regions of the country. For example, in Magdalena, a prevalence of 7.1% was recorded in analyzed canine patients (Thomas *et al.* 2020). In the Valle de Aburrá, it was identified as the primary canine tick-borne pathogen, with a higher prevalence than expected, suggesting that the infection might be underreported or underdiagnosed in the region (Cabrera–Jaramillo *et al.* 2022). In Cúcuta, an asymptomatic case was reported (Cala *et al.* 2018), and a study of dogs with clinical signs suspected of hemoparasitic infection revealed a positivity rate of 8.6% (Chinchilla *et al.* 2020). To date, no cases of *Hepatozoon americanum* have been reported in the country.

In a study conducted in the department of Magdalena, the detection of *Hepatozoon canis* by PCR was 7.1% in the canine patients examined (Thomas *et al.* 2020), a relatively low percentage. On the other hand, in the Valle de Aburrá, *H. canis* was observed to be the primary canine tick-borne pathogen, with a higher prevalence than expected, suggesting that it may be underreported or underdiagnosed (Cabrera–Jaramillo *et al.* 2022). Additionally, two PCR-confirmed studies have been reported in the city of Cúcuta, one of which identified a case with no clinical signs (Cala *et al.* 2018). In the same city, another PCR detection study found a positivity rate of 8.6% in dogs with clinical signs suspected of hemoparasitic infection (Chinchilla *et al.* 2020).

In this sense, the objective of this case report is to inform about the presence of *Hepatozoon canis* in the city of Bogotá and to describe the currently available diagnostic methods and treatment options for its management.

CASE DESCRIPTION

A one-year-old spayed female mixed-breed dog was presented for a general consultation the day after her adoption from a shelter. On the same day, rapid tests using immunochromatography for distemper and canine parvovirus were performed, with negative results. Additionally, the patient received flea and tick treatment (Afoxolaner), deworming (Milbemycin oxime), and a polyvalent vaccination (distemper, parvovirus, coronavirus, parainfluenza, hepatitis, adenovirus, and leptospira), as well as a rabies vaccine. Prior to adoption, the patient had been treated for a possible diagnosis of "kennel cough" (canine respiratory infectious complex) for one week, receiving antibiotics and nebulizations, although the specific medications used were not detailed.

Since her adoption, the patient exhibited a fearful demeanor, intolerance to exercise, nocturnal coughing episodes, and moderate pruritus in the cervical region, abdomen, and distal areas of all four limbs. On physical examination, a moderate non-productive cough reflex was noted, along with pale mucous membranes, abdominal organomegaly in the left mesogastric region (suspected splenomegaly), dyskeratosis on the right abdomen, and an X-shaped scar potentially related to a previous ovariohysterectomy. Additionally, hypotrichosis with crusting was observed in the ventral cervical region and on the hind limbs, along with poor skin and coat quality and a low body condition score (3/9) with a weight of 5 kg.

The differential diagnosis included canine respiratory infectious complex, hemoparasitism (ehrlichiosis, babesiosis, anaplasmosis), malnutrition, dermatophytosis, and mite dermatitis. As part of the initial diagnostic plan, blood samples were collected from the cephalic vein for a complete blood count, Alanine Transaminase (ALT), and creatinine. Blood chemistry analyses were processed using a semi-automatic Rayto RT-1904 CV, while the complete blood count with differentiated proteins was processed using an automated Vetplus 3500 hematology analyzer, with manual confirmation of the count and morphology. The results revealed normocytic normochromic anemia, erythrocyte agglutination, thrombocytopenia, and hyperproteinemia due to hyperglobulinemia (table 1).

The initial treatment consisted of administering doxycycline (10 mg/kg orally, every 12 hours for 21 days), prednisolone (0.5 mg/kg orally, every 12 hours for 8 days, then every 24 hours for an additional 8 days), and Mirrapel® oil (1.5 ml orally every 24 hours until further notice). It was recommended to supplement the diet with commercially available gastrointestinal care food and, after the resolution of coughing, to start weekly medicated baths with 3% chlorhexidine shampoo.

Subsequently, a real-time multiplex PCR with probes for the detection of hemoparasites (canine distemper, *Anaplasma* spp., *Hepatozoon* spp., *Ehrlichia* spp., and *Babesia* spp.) was performed, which tested positive for *Hepatozoon* spp. Based on this result, the treatment was supplemented with imidocarb dipropionate (5 mg/kg via intramuscular injection), preceded by subcutaneous administration of atropine (0.02 mg/kg), with the procedure repeated 15 days later.

Seven and fifteen days after the first injection, a follow-up complete blood count was performed. The results, detailed in table 1, showed improvement with resolution of anemia and thrombocytopenia. At the first follow-up, myelocytes and hyperproteinemia due to hyperglobulinemia persisted; however, these anomalies were resolved by the second follow-up. Clinically, the patient demonstrated complete resolution of the clinical signs and gained 600 grams over a two-week period. By the time of the second injection, her weight had increased to 6 kg, with a body condition score of 4/9 and no abnormalities noted during physical examination. At the final follow-up, conducted seven months after the initial treatment, the patient remained without abnormalities and achieved an ideal body condition with a weight of 7 kg.

RESULTS AND DISCUSSION

The initial approach to the patient was based on the high-risk history, clinical signs,

TABLE 1. Complete blood count tests performed on the patient (abnormal values are highlighted with an asterisk)

	Before diagnosis		First checkup		Second checkup		
Parameter	**Absolute value**	**Relative value**	**Absolute value**	**Relative value**	**Absolutevalue**	**Relative value**	**Reference ranges**
Erythrocytes	4.8*		7.4		6.8		5.5-8.5 x 10 ˆ 12/L
Hemoglobin	10.1*		15.6		16.2		12.0-188 g/dL
Hematocrite	31.8*		46.8		48.7		37.0-55.0 %
MCV	66.3		63.3		71.8		60.0-77.0 fL
MCH	21.0		21.1		23.9		19.5-24.5 pg
MCHC	31.8*		33.3		33.3		32.0-36 g/dL
RDW	12.9		13.5		13.8		11.0-16.0 %
Platelets	100*		243		206		200-500 x 10 ˆ 9/L
MPV	8.8		8.7		8.4		7.0-12.0 fL
Total protein	8.4*		8.5 *		6.8		6.0-7.5 g/dL
Albumin	2,6		2.76		2.5		2.4-3.9 g/dL
Globulins	5.8*		5.7 *		4.3		2.5-4.5 g/dL
Leukocytes	15.0		7.4		6.8		6.0-17.0 x 10 ˆ 9/L
Neutrophils	10050	67	3848	52 *	3445	53 *	3,000-11, 500 ul 60-77 %
Band neutrophils	0	0	0	0	0	0	< 300 ul 0 %
Lymphocytes	4350	29	2146	29	2665	41*	1,000-4, 800 ul 15-35 %
Monocytes	0	0*	1036	14 *	260	4	< 1,200 ul 2-7 %
Eosinophils	600	4	222	3	130	2	100-1,000 ul 2-6 %
Basophils	0	0	0	0	0	0	< 100 ul 0-1 %
Metamyelocytes	0	0	148 *	2 *	0	0	0 ul 0 %

Source: own elaboration.

and previous treatment. The initial basic examinations allowed for the quantification of blood cell lines, and following manual confirmation of cell counts and evaluation of the blood smear, it was possible to infer a low parasitemia due to the absence of hemoparasite gamonts in the blood smear. However, the positive confirmation for *Hepatozoon canis* by PCR indicated that the infection was present but not in a sufficient quantity to be visualized in a single blood smear.

Additional tests included serum measurement of ALT to assess active liver damage and creatinine to detect severe renal damage or severe dehydration. Both values were normal, allowing for appropriate treatment to be directed and differential diagnoses to be established, including hemoparasitism and canine respiratory infectious complex (including distemper). In the case of hemoparasitism, the main suspected infectious agents were ehrlichiosis, anaplasmosis, and babesiosis, as these are the most common in clinical practice.

Dermatological findings were likely attributed to nutritional deficiencies rather than to hepatozoonosis, with notable improvement observed following supplementation with fatty acids, multivitamins, and high-quality food. Regarding diagnosis, the increasing availability of PCR profiles and tests in laboratories, especially for hemoparasites, enabled the identification of this lesser-known disease in Bogotá, which was not initially considered in the differential diagnoses. Without the PCR technique, a definitive diagnosis would not have been possible, as no intracellular inclusions of gamonts were observed in neutrophils or monocytes in routine blood smears. However, in patients with high parasitemia, direct observation of the smear remains a rapid and cost-effective diagnostic tool. It is essential that in non-endemic areas, such as Bogotá, or in cold climates where it has not been reported, blood smear examination be included in each processed complete blood count as part of routine paraclinical evaluation.

Among the molecular tests available in Bogotá, various PCR variants are employed, including multiplex and simplex, end-point, isothermal, real-time, and real-time with probe PCR. The latter was utilized in this case due to its superior sensitivity and specificity (Chinchilla *et al.* 2020). It is important to note that PCR results are qualitative (positive or negative), which are useful for diagnosis but not applicable for quantitative monitoring of parasitemia.

The initial treatment was prescribed based on the clinical history and paraclinical test results, utilizing doxycycline, a broad-spectrum antibiotic (Plumb 2018), at a dosage that covered both the bacteria associated with canine respiratory infectious complex and the most common hemoparasite, *Ehrlichia canis* (Papich 2021). However, this antibiotic is ineffective against *Hepatozoon canis*. Prednisolone was administered as an anti-inflammatory to treat the affected respiratory tract and to manage the potential diagnosis of *Ehrlichia canis* infection. Although the literature advises against the use of corticosteroids in patients with hepatozoonosis (Ettinger *et al.* 2017), by the time the diagnosis was confirmed via PCR, the treatment with prednisolone had already concluded without observed adverse effects. Additionally, nutritional support with high-quality food and multivitamins was provided, which facilitated the patient's overall recovery.

Although the PCR technique identifies only the genus *Hepatozoon*, the absence of severe clinical signs and the possibility of co-infections suggested that the infection was most likely *Hepatozoon canis* (Eiras *et al.* 2007). For this reason, the most appropriate treatment for this condition was chosen, involving the administration of imidocarb dipropionate via the parenteral route. Prior to each injection, premedication with atropine was administered to mitigate potential side effects associated with imidocarb dipropionate (Papich 2021). No adverse reactions were observed during the treatment.

Following the diagnosis of hepatozoonosis, the therapy with doxycycline, which had been previously initiated, was continued to address the canine respiratory infectious complex, given the high likelihood of co-infection in the patient. The presence of coughing and thrombocytopenia is not a common sign of *Hepatozoon* infection, suggesting the possibility of co-infection with another undetected hemoparasite, as the literature well documents the concomitance of multiple tick-borne pathogens (Tuna *et al.* 2021), particularly in patients affected by *Rhipicephalus sanguineus* (Greene 2012). Although no ticks were found during the initial clinical examination, recent treatment with afoxolaner may have eliminated any visible evidence of infestation. Nevertheless, prior contact with ticks cannot be ruled out and remains a key risk factor for the transmission of hemoparasites, as documented in studies identifying the tick species involved in such cases (Arcila *et al.* 2005).

Since no gamont forms of the hemoparasite were detected in the blood smears, a complete recovery cannot be confirmed, as there was no initial positive smear to serve as a reference. However, the success of the treatment can be evaluated based on the clinical improvement of the patient and the resolution of anemia and thrombocytopenia observed in the follow-up complete blood count (Mastrantonio *et al.* 2023). Although quantitative real-time PCR was not performed for follow-up, clinical and paraclinical improvement suggests that this technique could be a useful tool for monitoring treatment effectiveness if needed.

Preventing hepatozoonosis in companion animals presents various challenges due to its easy transmission through ectoparasites. To mitigate the risk of infection, it is essential to reduce exposure to endemic areas with poor vector control and to apply acaricides regularly to both animals and their environments, especially in locations with high animal concentration such as shelters, schools, and kennels (Beugnet *et al.* 2018). Veterinarians play a crucial role, not only in the early detection of the disease but also in implementing tailored preventive strategies adapted to the risk profile of each patient or group. Effective prevention extends beyond the use of antiparasitic drugs, requiring constant vigilance and owner education to ensure long-term animal welfare.

CONCLUSION

Hepatozoonosis is not commonly considered a differential diagnosis among tick-borne diseases in Bogotá, leading to an underdiagnosis of this condition, particularly in animals from shelters with uncertain origins. It is possible that both dogs and cats, as well as the ticks infesting them, may have been transported from endemic areas, and climate change may facilitate the survival of these ectoparasites in previously cooler climates, increasing the risk of infection in urban areas such as Bogotá.

Given that the goal of therapy is not the complete eradication of the parasite but rather the resolution of clinical signs, the treatment was successful according to the literature. Two of the reported medications for treating *Hepatozoon* sp. infection and its possible coinfections were used, suggesting, given the good clinical response, that the infection was likely *Hepatozoon canis.* However, it is important to develop tests that can identify whether the infection is caused by *H. canis* or *H. americanum*, as both species exhibit differences in clinical presentation and treatment, and there is a possibility that both may emerge in the country.

Finally, it is crucial to conduct studies that assess the significance of hepatozoonosis as an emerging disease in Bogotá. The increasing mobility of dogs between cities and the potential for vertical transmission highlight the importance of further investigating the epidemiology of this disease in the country.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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USE OF ARTIFICIAL INTELLIGENCE

No artificial intelligence was used during the diagnostic and treatment process, nor in the preparation of this manuscript.

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