Clinical and hematological signs associated with dogs naturally infected by *Hepatozoon* sp. and with other hematozoa: A retrospective study in Uberlândia, Minas Gerais, Brazil

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Abstract

An evaluation was made of the clinical and hematological aspects of 115 dogs infected naturally by *Hepatozoon* sp. and treated at the Hospital School of the Faculty of Veterinary Medicine in Uberlândia, MG, Brazil. Of the 115 dogs for whom peripheral blood films were evaluated, 89 (77.39%) presented parasitemia by *Hepatozoon* sp. solely, while 26 (22.61%) had combination of *Hepatozoon* sp., *Babesia* sp. and *Ehrlichia* sp. Young male dogs less than a year old, of undefined breed (UB), were the most commonly affected. Thirty-nine (33.92%) of the dogs were asymptomatic while 76 (66.08%) presented varied clinical symptoms, the most frequent being anorexia, pulmonary alterations, hyperthermia, pale mucosae, apathy and/or prostration, and diarrhea. The majority of hematological alterations were normochromic-normocytic anemia, leukocytosis, neutrophilia, and nuclear deviation of neutrophils to the left (NDNL). The findings of this study confirm that *Hepatozoon* sp. causes clinical and hematological alterations of varied intensity, which, albeit not specific to canine hepatozoonosis, reinforce the notion that the discovery of the agent in dogs, even with low parasitemia, should be taken into consideration.

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1. Introduction

Canine hepatozoonosis is a disease transmitted by ticks and caused by the protozoan *Hepatozoon* of the phylum Apicomplexa of the Family Hepatozoidae, also described in other carnivores of various regions around the world. *Hepatozoon* gamonts isolated from dogs in distinct geographic regions are morphologically similar and, until 1997, it was believed that canine hepatozoonosis was caused by a single species (Baneth et al., 2003). However, subsequent research has revealed that dogs are infected by two species—*Hepatozoon canis* and *Hepatozoon americanum*. Albeit phylogenetically related, these protozoa are distinct in their clinical, pathological, genetic, and antigenic aspects and in regard to their transmission vector (Mathew et al., 2000).
The species occurring in Brazil is *H. canis*, which is prevalent in several regions of the country, considered to have a low pathogenicity and often found in association with other infections (O’Dwyer et al., 2004; Rubini et al., 2005). However, Forlano et al. (2007) suggest that so far it cannot be stated that only the species *H. canis* occurs in Brazil, and that further studies are necessary to characterize the disease in dogs and confirm the occurrence or absence of other species of the parasite.

The brown dog tick *Rhipicephalus sanguineus* has been described as a vector for *H. canis*, although other species such as *Amblyomma ovale* are considered potential transmitters (Baneth et al., 2003; Forlano et al., 2005).

Infection by *H. canis* varies from asymptomatic cases in apparently healthy dogs to severe and potentially fatal cases of the disease that cause fever, cases of paralysis, anorexia, emaciation, anemia, ocular discharge, and hind leg weakness, which are characteristic symptoms of the disease (Mundim et al., 1994; Gondim et al., 1998). The mild form of the disease is associated to low parasitemia, while severe signs are found in dogs with high parasitemia, often with close to 100% of their neutrophils affected by the parasite (Baneth et al., 2003).

The abnormalities commonly found in laboratory analyses are anemia, leukocytosis, neutrophilia, and augmented alkaline phosphatase (Baneth et al., 2003).

In hepatozoonosis caused by *H. canis*, clinical and laboratory findings are often masked by the presence of concomitant infections and such findings should not be attributed exclusively to *H. canis*.

The purpose of this study is to retrospectively describe the clinical and hematological findings of dogs naturally infected only by *Hepatozoon* and with infections associated to other agents, in view of the paucity of information about the disease in our region of the world.

2. Materials and methods

This study involved 115 dogs of both sexes, of different breeds and ages. The dogs, which displayed varied clinical symptoms, were attended at the Hospital School of the Federal University of Uberlândia’s Faculty of Veterinary Medicine in Uberlândia, state of Minas Gerais, Brazil, from 1995 to 2005. The dogs were diagnosed as naturally infected by *Hepatozoon* sp., based on the gametocytes found inside the leukocytes in blood films, which were prepared with blood taken from marginal ear veins and stained by the May-Grünwald-Giemsa method.

For the hemograms, 2 mL of blood was collected from each animal by cephalic vein puncture, using disposable needles and syringes. The blood samples were stored in sterile tubes containing 0.1 mL of 10% EDTA K3 (tripotassic EDTA) solution. The globular volume was determined by the microhematocrit method (Fanem model 211 microcentrifuge), the hemoglobinometry by the cianometahemoglobin method (Celm HB-520 hemoglobinometer), and hematimetry and leukometry were carried out in an automatic blood cell counter (Celm CC 510). Differential or specific leukocyte counts were done on blood films stained by the May-Grünwald-Giemsa method (Ferreira Neto et al., 1982). The values of the erythrogram and leukogram parameters were compared with the reference values as described by Meinkoth and Clinkebeard (2000).

The parasitemia rate was calculated directly from the blood films, which were prepared with the blood samples taken from the marginal ear veins, as described by Gavazza et al. (2003), and a count was made of the number of infected neutrophils and monocytes in 200 leukocytes.

The data on age, sex, clinical manifestations, and concomitant infections were obtained from the animals’ clinical histories filled out during the consultation, placed on charts and analyzed statistically.

The data were analyzed using descriptive statistics (mean, standard deviation, minima, and maxima) and the binomial nonparametric test for two proportions, considering a 5% level of significance. The analyses were carried out with SAS software (SAS, 2005).

3. Results

With regard to age, it was found that 39 (33.91%) of the 115 dogs evaluated were less than 1-year-old, and the remainder were distributed in several age groups up to 10 years old. Samples were collected from 69 (60%) males and 46 (40%) females, which was a statistically significant difference (*P* < 0.05).

Sixty-four (55.65%) of the dogs were of undefined breed (UB) and 51 (44.34%) were of 16 different breeds. Among the purebreds, the Brazilian mastiff represented the highest proportion of infected dogs (6.69%).

Of the 115 naturally infected dogs, 89 (77.39%) were infected only by *Hepatozoon* sp. and 26 (22.61%) showed concomitant infection by *Ehrlichia* sp. and *Babesia* sp. (*Table 1*).

Seventy-six (66.08%) of the dogs displayed some clinical sign and the blood films showed that of these, 50
were infected only by *Hepatozoon* sp. and 26 (34.21%) were infected concomitantly by *Ehrlichia* sp. and/or *Babesia* sp. The 39 remaining dogs were asymptomatic, showing only *Hepatozoon* sp. in their blood films.

As for parasitemia, 112 dogs presented 0.1–2% of infected leukocytes, allowing them to be classified as low parasitemia, while three dogs showed 10–30% of infected leukocytes and were therefore considered of medium parasitemia.

Anorexia, hyperthermia, diarrhea, vomiting, pulmonary and respiratory alterations, pale mucosae, apathy and/or prostration were common clinical symptoms and were more severe both in the animals with a single infection and in those with parasitemia associated with other hematozoa (Table 2).

Five of the 115 dogs died, three of them infected only by *Hepatozoon* and two infected concomitantly with *Ehrlichia* sp.

The values of the erythrogram and leukogram parameters of the animals of this study varied widely. The mean erythrocyte, hemoglobin and hematocrit counts were lower than the reference values (Meinkoth and Clinkebeard, 2000), while the leukocytes, total and band neutrophils were higher (Table 3).

The hematological alterations consisted mainly of anemia (70.43%), leukocytosis (39.13%), neutrophilia (48.70%), nuclear deviation of neutrophils to the left (NDNL) (82.61%) and eosinopenia (40.87%).

### Table 2

Clinical signs observed in the 76 dogs naturally infected by *Hepatozoon* sp., in a single infection or in association with other hematozoan, Uberlandia, MG, Brazil

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Animals</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia and loss of appetite</td>
<td>56</td>
<td>73.68 a</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>49</td>
<td>64.47 a</td>
</tr>
<tr>
<td>Diarrhea (bloody, in some cases)</td>
<td>39</td>
<td>51.31 a</td>
</tr>
<tr>
<td>Vomiting</td>
<td>33</td>
<td>43.42 b</td>
</tr>
<tr>
<td>Locomotor alterations of various degrees</td>
<td>27</td>
<td>35.53 c</td>
</tr>
<tr>
<td>Hyperemia of mucosae</td>
<td>21</td>
<td>27.63 cd</td>
</tr>
<tr>
<td>Pale mucosae</td>
<td>45</td>
<td>59.21 a</td>
</tr>
<tr>
<td>Apathy and/or prostration</td>
<td>40</td>
<td>52.63 ab</td>
</tr>
<tr>
<td>Serous, mucous, or mucopurulent nasal discharge</td>
<td>25</td>
<td>32.89 c</td>
</tr>
<tr>
<td>Ocular discharge</td>
<td>26</td>
<td>34.21 b</td>
</tr>
<tr>
<td>Pulmonary and respiratory alterations</td>
<td>53</td>
<td>69.73 a</td>
</tr>
<tr>
<td>Cardiac alterations</td>
<td>25</td>
<td>23.89 d</td>
</tr>
<tr>
<td>Enlarged spleen sensitive to touch</td>
<td>22</td>
<td>28.94 cd</td>
</tr>
<tr>
<td>Hepatic region sensitive to touch</td>
<td>22</td>
<td>28.94 cd</td>
</tr>
<tr>
<td>Kidney region sensitive to touch</td>
<td>28</td>
<td>36.84 b</td>
</tr>
<tr>
<td>Weight loss and cachexia</td>
<td>26</td>
<td>34.21 c</td>
</tr>
<tr>
<td>Dehydration of varied degrees</td>
<td>19</td>
<td>25.00 d</td>
</tr>
<tr>
<td>Enlarged lymphonodes</td>
<td>14</td>
<td>18.42 d</td>
</tr>
</tbody>
</table>

Proportions followed by the same letter in the column do not differ statistically by the binomial test for two proportions, considering a significance of 0.05.

### Table 1

Proportion of infested animals among 115 domestic dogs infected with *Hepatozoon* sp. and other hematozoan species, Uberlandia, MG, Brazil

<table>
<thead>
<tr>
<th>Hematozoan species</th>
<th>Infested animals</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hepatozoon</em> sp.</td>
<td></td>
<td>89</td>
<td>77.39 a</td>
</tr>
<tr>
<td><em>Hepatozoon</em> sp. + <em>Ehrlichia</em> sp.</td>
<td></td>
<td>20</td>
<td>17.39 b</td>
</tr>
<tr>
<td><em>Hepatozoon</em> sp. + <em>Babesia</em> sp.</td>
<td></td>
<td>02</td>
<td>1.74 c</td>
</tr>
<tr>
<td><em>Hepatozoon</em> sp. + <em>Ehrlichia</em> sp. + <em>Babesia</em> sp.</td>
<td></td>
<td>04</td>
<td>3.48 c</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>115</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Proportions followed by different letters differ statistically by the binomial test for two proportions, considering a significance of 0.05.

(65.79%) were infected only by *Hepatozoon* sp. and 26 (34.21%) were infected concomitantly by *Ehrlichia* sp. and/or *Babesia* sp. The 39 remaining dogs were asymptomatic, showing only *Hepatozoon* sp. in their blood films.

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The hematological alterations consisted mainly of anemia (70.43%), leukocytosis (39.13%), neutrophilia (48.70%), nuclear deviation of neutrophils to the left (NDNL) (82.61%) and eosinopenia (40.87%).

### 4. Discussion

Despite advances made in the diagnosis of hemoparasitoses in recent years, the methodology employed in this study is the same as that used in the hospital where the study was conducted. It was not possible to state that only Hepatozoon caused disease or that the animals were not previously coinfected with other agents. The parameters evaluated were the ones the animals presented when they were examined and material was collected. Subclinical infections can only be identified through molecular biology.

Canine hepatozoonosis is a disease diagnosed in various regions of Brazil, and *H. canis* is the species recognized as causing the infection (O’Dwyer et al., 2004; Forlano et al., 2007), which affects dogs of all ages. In this study we evaluated the behavior of the infection, and the most frequent symptoms and hematological alterations.

The presence of the parasite, especially in dogs up to the age of 1 year, corroborates the statement that animals of all ages can become infected, although it is prevalent among younger dogs (Mundim et al., 1994; Gavazza et al., 2003). This prevalence may be ascribed to the low immunity of young animals whose immune system is not yet fully developed.

Infection by this protozoan in a significantly higher proportion of male dogs reinforces the findings of Gavazza et al. (2003), who reported a higher prevalence of infection among males. Beaufils and Martin-Granel (1988), however, stated that there was no predisposition of sex for this infection. The higher occurrence of males in this study was probably due to the predominance of male dogs in the group under study, or it may be related
with their higher exposure to ticks, the vector of the disease, due to behavioral habits.

In this study, the larger proportion of parasitism among cross-breed dogs was due to their higher proportion among the dogs attended at the Hospital School. The same explanation holds true for the Brazilian Mastiff, since it is the animal of choice for guarding homes due to its physical and behavioral traits.

Hepatozoonosis does not show a predisposition for any particular breed, but is strongly associated to behavioral characteristics that favor the animal’s contact with the vector, increasing the possibility of transmission and infection.

Hepatozoonosis is often found in association with other infections (O’Dwyer et al., 1997; Gondim et al., 1998), especially with other hematozoa commonly observed in dogs, such as *Ehrlichia* and *Babesia*. In this study, 26 animals had concomitant infections, and *Ehrlichia* was the agent most frequently found in association with *Hepatozoon* sp. The association of *Hepatozoon* sp. with other hematozoa can be attributed to the presence of the vector, the tick *R. sanguineus*, which is also a transmitter of *Ehrlichia* and *Babesia* (Gondim et al., 1998; O’Dwyer et al., 2001). Experimental studies confirm that *A. ovale* is a potential vector of *Hepatozoon* spp. in Brazil (Forlano et al., 2005).

The asymptomatic dogs in this study presented a low number of circulating leukocytes infected with gametocytes, as did those that exhibited a variety of mild clinical signs. According to Baneth and Weigler (1997), the severity of clinical signs and the parasitemia rate are correlated. The agent can be detected in leukocytes of clinically healthy dogs (Vincent-Johnson et al., 1997; Baneth et al., 2003). Highly infected animals show more severe systemic manifestations than dogs with low parasitemia (Baneth et al., 1995; Baneth and Weigler, 1997; Gavazza et al., 2003). The symptomatology of dogs with *H. canis* varies from mild or even asymptomatic to severe signs, depending on the parasitemia and the animal’s immune state. The disease is more common in its mild form since low parasitemia is more frequent (subclinical condition). The clinical findings of this study corroborate those of other researchers (Craig et al., 1978; Baneth and Weigler, 1997; Panciera et al., 1997; Vincent-Johnson et al., 1997; Gondim et al., 1998; Gavazza et al., 2003; Assarasakorn et al., 2006).

There are diverging opinions regarding the pathogenicity of *H. canis* and the opportunistic nature of this parasite. The presence of clinical signs, even mild ones, is congruent with the statement of Baneth et al. (2003), who allege that infection by *Hepatozoon* induces the appearance of moderate or unapparent disease and is therefore nonpathogenic. The pathogenic aspect of Hepatozoon was also mentioned by Gavazza et al. (2003), who detected dogs with infection only by *H. canis* with clinical signs of the disease. These authors concluded that the alterations they detected were directly related to infection and considered it the primary pathogen causing the disease. Baneth and Weigler (1997),

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>S.D.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC(×10⁶ cells/μL)</td>
<td>4.77</td>
<td>1.44</td>
<td>1.21</td>
<td>8.61</td>
<td>5.50–8.50</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.93</td>
<td>3.18</td>
<td>2.00</td>
<td>18.20</td>
<td>12.00–18.00</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>30.61</td>
<td>9.62</td>
<td>8.00</td>
<td>55.00</td>
<td>37.00–55.00</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>64.00</td>
<td>3.34</td>
<td>55.56</td>
<td>75.57</td>
<td>60.00–77.00</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.84</td>
<td>1.31</td>
<td>16.53</td>
<td>25.36</td>
<td>19.00–23.00</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.44</td>
<td>1.48</td>
<td>25.00</td>
<td>35.00</td>
<td>32.00–36.00</td>
</tr>
<tr>
<td>WBC (cells/μL)</td>
<td>20,900</td>
<td>24,089</td>
<td>600</td>
<td>146,000</td>
<td>6,000–17,000</td>
</tr>
<tr>
<td>Band neutrophils (cells/μL)</td>
<td>3,706</td>
<td>6,547</td>
<td>0</td>
<td>33,228</td>
<td>0–300</td>
</tr>
<tr>
<td>Segmented neutrophils (cells/μL)</td>
<td>13,451</td>
<td>16,511</td>
<td>288</td>
<td>108,000</td>
<td>3,000–11,500</td>
</tr>
<tr>
<td>Total neutrophils (cells/μL)</td>
<td>17,165</td>
<td>21,531</td>
<td>426</td>
<td>127,194</td>
<td>3,000–11,800</td>
</tr>
<tr>
<td>Lymphocytes (cells/μL)</td>
<td>2,574</td>
<td>2,925</td>
<td>156</td>
<td>27,120</td>
<td>1,000–4,800</td>
</tr>
<tr>
<td>Eosinophils (cells/μL)</td>
<td>565</td>
<td>835</td>
<td>0</td>
<td>4,658</td>
<td>100–1,250</td>
</tr>
<tr>
<td>Monocytes (cells/μL)</td>
<td>576</td>
<td>792</td>
<td>0</td>
<td>5,848</td>
<td>150–1,350</td>
</tr>
<tr>
<td>Basophils (cells/μL)</td>
<td>3</td>
<td>27</td>
<td>0</td>
<td>276</td>
<td>Rare</td>
</tr>
</tbody>
</table>

RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cells.

a S.D. = standard deviation.

b Meinkoth and Clinkebeard (2000).
Weigler (1997) and Voyoda et al. (2004) consider that the finding of parasitemia by this agent should not be discarded and should be analyzed clinically, for there are differences in the hematological and body temperature parameters between dogs testing positive for *H. canis* and healthy dogs. Dogs can have concomitant infections by agents of bacterial or viral origin, such as monocytic ehrlichiosis and cynomosis, which lower their immune defense, exacerbating the clinical signs of these infections (Elias and Homans, 1988; Voyoda et al., 2004).

The variations observed in the erythrogram and leukogram parameters of the dogs in this study (Table 3) may be related to parasitemia, multiplication of the protozoan in the animals' tissues, the capacity of response of each animal and concomitant infection by other infectious agents. As Bansal et al. (1985) and Gosset et al. (1985) stated, the hematological findings are altered when hepatozoonosis is associated to concomitant infections.

The anemia observed in 81 dogs of this study may be associated with the chronicity of the infection, as well as with concomitant infection by *Ehrlichia* sp. and/or *Babesia* sp. and with the occurrence of bloody diarrhea in some of the animals, in line with the findings of Beaufils and Martin-Granel (1988). For some researchers, anemia is common in infections by *H. canis* (Baneth et al., 1995; Gondim et al., 1998; O’Dwyer et al., 2006). The presence of normocytic normochromic anemia in most of the infected dogs is a finding consistent with those of Craig et al. (1978), Mundim et al. (1992), Mancitire et al. (1997) and Gavazza et al. (2003).

The NDNL, neutrophilia and leukocytosis in the animals of this study are consistent with the findings of other researchers, who stated these alterations are frequent (Mundim et al., 1992; Mancitire et al., 1997; Voyoda et al., 2004). However, these results differ from those of Barton et al. (1985), Elias and Homans (1988), Baneth et al. (1995) and Mancitire et al. (1997), who found neutropenia and stated that NDNL is rarely observed. The alterations observed here may be due to the parasite’s invasion and multiplication in the animal’s tissues and organs, leading to an inflammatory response exacerbated by secondary bacterial infections intercurrent with other hematozoa. Gaunt et al. (1983) stated that anemia and neutrophilia are probably secondary to necrosis and inflammation of the spleen, lymphonodes, liver and lungs.

Eosinopenia observed in the animals infected is not congruous with the findings of Gaunt et al. (1983), Beaufils et al. (1996) and Gavazza et al. (2003), who observed eosinophilia. This response may be explained by the destruction of this type of cell by corticosteroids and catecholamines released by the organism during the stress caused by the disease.

Although the platelet count is an important parameter in the evaluation of frequent hematological abnormalities caused by *Ehrlichia, Babesia* and *Hepatozoon*, this count was not done for all the dogs for operational reasons; we therefore decided not to include it in this study.

These clinical and hematological findings, albeit not specific for canine hepatozoonosis, reinforce the hypothesis that the presence of this agent in dogs should be taken into account even when parasitemia is low.

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**References**


