

Clinical and blood count findings in dogs naturally infected with *Dirofilaria immitis*

Achados clínicos e de hemograma em cães naturalmente infectados por *Dirofilaria immitis*

Alexandre José Rodrigues Bendas^{1*}, Bruno Alberigi¹, Suzane Galardo², Norma Labarthe³ & Flavya Mendes-de-Almeida⁴

¹ Veterinarian, DSc. Departamento de medicina e cirurgia veterinária (DMCV), Instituto de Veterinária (IV), Universidade Federal Rural do Rio de Janeiro (UFRRJ). Campus Seropédica, RJ, Brazil.

² Veterinarian, Autonomus, Rio de Janeiro, RJ, Brazil

³ Veterinarian, DSc. Programa de Pós-Graduação Bioética, Ética Aplicada e Saúde Coletiva (PPGBios), Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

⁴ Veterinarian, DSc. Departamento de Patologia e Clínica Veterinária (MCV), Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil.

Abstract

Dirofilaria immitis is a nematode that infects canids worldwide as well as other mammalian species, including humans. Worms and dogs are well adapted to one another, making dogs the best urban host for the parasite. Nevertheless, 30% of dogs do not sufficiently present microfilaremia, that is, the low larval load impairs transmission by mosquitoes and diagnosis by its detection in the blood samples. Therefore, the canine diagnosis must always include a microfilaria test and serological tests to detect adult worm antigens. To describe the clinical findings in naturally infected dogs in Rio de Janeiro, 34 dogs were included in the study. All dogs were evaluated for history, anamnesis, physical examination, complete blood count (CBC), *D. immitis* testing for antigens (ELISA test SNAP 4Dx Plus®), and microfilarial burden. The most frequent complaint from the owners was coughing (14.7%, 5/34). The most common CBC finding was eosinophilia (29.4%), followed by thrombocytopenia (26.5%) and neutrophilia (14.7%). Of the 34 animals, 91.2% were microfilaremic, with a mean count of 11.939 microfilaria/mL. Veterinarians working in areas endemic to *D. immitis* should always undergo screening tests and pulmonary auscultation, and increased expiratory sounds, even in the absence of coughing, can be considered a sign of the disease, along with eosinophilia, thrombocytopenia, and neutrophilia.

Keywords: heartworm, clinical signs, complete blood count, lung inflammation.

Resumo

Dirofilaria immitis, é um nematoide que infecta canídeos em todo o mundo, bem como outras espécies de mamíferos, incluindo humanos. Os vermes e os cães estão bem adaptados um ao outro, tornando os cães o melhor hospedeiro urbano para o parasita. Contudo, 30% dos cães não apresentam microfilaremia, prejudicando a transmissão pelos mosquitos e o diagnóstico por detecção de larvas em amostras de sangue. Portanto, o diagnóstico canino deve incluir sempre a pesquisa de microfílaras e sorologia para detecção do antígeno do verme adulto. Com o objetivo de descrever os achados clínicos de cães naturalmente infectados no Rio de Janeiro, 34 cães foram incluídos. Todos os cães foram avaliados por histórico, anamnese, exame físico, hemograma completo (CBC), teste de antígenos, pesquisa e contagem de microfílaras de *D. immitis*. A queixa mais frequente dos responsáveis foi a tosse (14,7% - 5/34). O achado de hemograma mais comum foi eosinofilia (29,4%), seguido de trombocitopenia (26,5%) e neutrofilia (14,7%). Dos 34 animais, 91,2% eram microfilarêmicos com contagem média de 11.939 microfílaras/mL. Os veterinários que atuam em áreas endêmicas de *D. immitis* devem sempre realizar exames de triagem e ausculta pulmonar, pois mesmo na ausência de tosse, sons expiratórios aumentados podem ser considerados um sinal da doença, assim como eosinofilia, trombocitopenia e neutrofilia.

Palavras-chave: verme do coração, sinais clínicos, hemograma, inflamação pulmonar.

Introduction

Dirofilaria immitis is a nematode that infects canids worldwide as well as other mammalian species, including humans. The parasite-canid relationship allows dogs to harbor large numbers of adult parasites and keep the larvae circulating in their bloodstream for as long as 2 years



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*Correspondence

Alexandre José Rodrigues Bendas
Departamento de Medicina e Cirurgia
Veterinária - DMCV, Instituto de Veterinária
- IV, Universidade Federal Rural do Rio de
Janeiro - UFRRJ
Rodovia BR 465, Km 7, Campus Universitário,
Zona Rural
CEP 23897-000 - Seropédica (RJ), Brasil
E-mail: alexandre bendas@gmail.com

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(Abraham, 1988). Although well adapted to one another, 30% of dogs do not sufficiently present microfilaremia (Nelson et al., 2014), thus impairing diagnosis by the detection of larvae in blood samples. Therefore, the canine diagnosis must always include the detection of microfilariae by a concentration method and serology test to detect adult worm antigens.

Many pathophysiological mechanisms are associated with heartworm disease, including cardiopulmonary abnormalities associated with parenchymal lung disease and right heart overload (Tudor et al., 2014). The clinical signs of canine heartworm disease can vary greatly, from asymptomatic to severe, and even death. Clinical signs develop gradually and usually begin with decreased exercise tolerance, a chronic cough that progresses to moderate to severe dyspnea, prostration, ascites, cachexia, and post-exercise syncope or excitement (Venco et al., 2005). Animal death usually occurs because of right-sided heart failure (Atwell et al., 1988).

The most frequently reported hematological findings in dogs with *D. immitis* are thrombocytopenia, non-regenerative anemia, leukocytosis by neutrophilia, eosinophilia, and basophilia (Oliveira et al., 2013; Polizopoulou et al., 2000; Rocha, 2010). Thrombocytopenia found in infected dogs may be the result of immune-mediated platelet destruction (Waner et al., 1995) or platelet consumption by the pulmonary arterial system (Nelson & Couto, 2015). Anemia of inflammation is usually characterized by mild to moderate non-regenerative anemia associated with neutrophilic leukocytosis, hypoferrremia, and increased storage of iron in tissues (Bezerra et al., 2021). Eosinophilia and basophilia are commonly associated with allergic and parasitic infections (Lilliehöök et al., 2000). Neutrophilia, lymphopenia, monocytosis, and eosinophilia can be caused by chronic stress from the parasite and leukogram of stress (Scott, 2008).

Although most dogs are asymptomatic, laboratory findings may be present; however, the nonspecific clinical signs contribute to a delay in diagnosis and consequently to the worsening of the condition. Since clinical presentation, particularly complete blood count (CBC), is known to vary according to local conditions, a clinical description of the heartworm of naturally infected dogs in Rio de Janeiro is presented to increase the local small animals' practitioner's awareness and facilitate the inclusion of *D. immitis* infection in the differential diagnosis.

Material and methods

The use of animals in this study was approved by the Ethics Committee on the Use of Animals of the Universidade Federal Fluminense (No. 499).

Dogs with *D. immitis* infection that presented at a private hospital in Rio de Janeiro were eligible for enrollment. To be included in the study, dogs had to be on preventive medications in the last 6 months, tested positive on the *D. immitis* antigen confirmatory test (ELISA test SNAP 4Dx Plus® according to the manufacturer's recommendations), and their owners must have signed the informed consent term presented.

All the animals included in the study had a sequentially numbered data capture form. In addition to history and anamnesis details, thorough physical examination findings using routine cardiorespiratory system procedures (Feitosa, 2014) and CBC were performed using an automated blood cell counter (ABX-ABCvet), and microfilariae test results were recorded. Indices of red blood cells, morphological differences, and counting were performed manually, as described by Jain (1986).

Microfilariae were detected by whole blood smears prepared for CBC or by the concentration test of Knott (1939), modified by Newton and Wright (1956). Microfilariae were counted blindly by the same two operators using two stained slides of 20 µL of whole blood. The final result was the arithmetic mean of the four countings multiplied by 50. The concentration of the microfilariae was adjusted to 1 mL by multiplying the final result of each sample by 50 (Bendas et al., 2008).

Data on history and anamnesis, physical examination of the cardiorespiratory system, CBC, and microfilariae tests were collected to complete the data capture form (supplementary data). The data obtained were analyzed using mean and standard deviation.

Results

A total of 34 *D. immitis* antigen-positive dogs were evaluated. All the dogs mostly lived indoors. Among them, 12 were male (4 neutered) and 22 were female (11 spayed), aged between 2 to 8 years, and weighed between 2.5 kg and 47.8 kg (\bar{x} : 18.76 kg, \pm 10.6).

According to the owners' information, 26.5% of the dogs (9/36) presented signs of heartworm disease unrelated to physical activity or time of the day. Coughing (14.7%, 5/34) was the only complaint reported by the owners. None of the owners reported any events of exercise intolerance, dyspnea at rest, cyanosis, syncope, or convulsion.

On physical examination, none of the dogs presented with a positive tracheal cough reflex, cyanosis, dehydration, abnormal abdominal palpation, arterial pulse, or chest percussion.

The most frequent physical abnormality detected was continuous expiratory lung sounds (41.17%, 14/34). All animals presented with regular cardiac rhythm. The only abnormality detected during cardiac auscultation was a systolic murmur at the tricuspid focus (14.7%, 5/34).

CBC was within the normal range in 26.5% of the dogs (9/34). The most frequent deviation detected was eosinophilia (29.4%, 10/34), followed by thrombocytopenia (26.5%, 9/34), and neutrophilia (14.7%, 5/34). One dog was diagnosed with lymphoid leukemia based on the blood count (Table 1).

Table 1. Hematological findings in dogs naturally infected with *Dirofilaria immitis* (n) in the city of Rio de Janeiro, Brazil.

Hematological findings	n/total	%
Thrombocytopenia	4/34	11.8
Thrombocytopenia with neutrophilia	1/34	2.9
Thrombocytopenia with neutrophilia and monocytosis	1/34	2.9
Eosinophilia	6/34	17.6
Eosinophilia with the presence of thrombocytopenia and normocytic normochromic nonregenerative anemia	1/34	2.9
Eosinophilia with neutrophilia and thrombocytopenia	1/34	2.9
Eosinophilia with neutrophilia	1/34	2.9
Eosinophilia with normocytic normochromic nonregenerative anemia, neutrophilia, monocytosis, and thrombocytopenia	1/34	2.9
Lymphopenia	2/34	5.9
Reactive lymphocytes	3/34	8.8
Normocytic normochromic nonregenerative anemia	1/34	2.9
Normocytic normochromic nonregenerative anemia with the presence of activated monocytes	1/34	2.9
Lymphoid Leukemia	1/34	2.9
Monocytosis	1/34	2.9
Within normal range	9/34	26.5

Microfilariae were detected in only 23.5% (8/34) of the blood smears prepared for CBC, whereas 91.2% (31/34) of the dogs were microfilaremic, according to the modified Knott's test. Among the preparations for microfilariae, only 20 (58.8%) presented with microfilariae. Average microfilaremia was 11.939 microfilariae/mL, ranging from 437 to 44.537 microfilariae/mL.

Discussion

Although *D. immitis* can infect dogs of any age, all the dogs included in this study were over 2 years old. This may be owing to the long life cycle of the parasite, which impairs early diagnosis

(Nelson et al., 2014) as well as the chronic character of the disease that may veil clinical signs during the early stages (Polizopoulou et al., 2000). The lack of clinical signs in the early stages may mislead owners and practitioners leading to mistakenly postponing screening tests.

Interestingly, the most frequent clinical sign of canine heartworm disease is exercise intolerance (approximately 46% of infected dogs) (Polizopoulou et al., 2000), which was not reported in the 34 dogs examined. Although owners complained of coughing, a well-recognized sign of heartworm disease (Nelson et al., 2014; Polizopoulou et al., 2000), no dog presented with a positive tracheal cough reflex. The lack of positive tracheal cough reflex and exercise intolerance suggests that the disease was in its initial stages because these signs point to the chronicity of the disease. As the cough reported by the owners was not confirmed by the tracheal cough reflex and with the possibility of the disease being in its initial stage, it may be inferred that the cough receptors were not activated during the physical examination of these animals (Feitosa, 2014), reinforcing the staging of the disease.

The continuous expiratory lung sounds detected in almost half of the dogs examined (41.2%) suggest that the inflammatory process imposed by the presence of worms in pulmonary arterioles (Genchi et al., 2012) was in progress and that it started immediately after worms' arrival to the lungs. This inflammation involves the lung parenchyma and progresses to the airways rather quickly, particularly because the disease in all dogs seemed to have been in its early stages by the time they were examined. The inflamed airway presents a reduced lumen, which increases air velocity, causing vortices and producing a louder sound that can be detected by auscultation (Knight, 2004). Therefore, canine auscultation in endemic areas must be performed carefully once it provides the first signal for the need for further investigation.

The absence of rhythm impairment observed in all dogs was expected because arrhythmias are a rare clinical sign in heartworm-infected dogs (Lombard & Ackerman, 1984). Contrastingly, the tricuspid murmur was detected in 14.7% of the dogs, suggesting that although the disease seemed to be in its early stages, some degree of right cardiac overload could be ongoing or that adult worms could be physically interfering with the correct coaptation of the leaflets (Romano et al., 2021).

The CBC showed that the blood cell count tended to be moderately changed or even unchanged. Since eosinopenia may be a sign of acute infection and eosinophilia is known to be related to chronic infections, even more so in presence of pulmonary involvement (Lilliehöök et al., 2000), the eosinophilia observed may be considered as the confirmation of the chronicity of the disease and the pulmonary reaction to the presence of worms. Thrombocytopenia can be related to the immune-mediated destruction of platelets or the increased activity of thrombocytes during heartworm infection (Niwetpathomwat et al., 2007).

The presence of neutrophilia, monocytosis, and activated monocytes, although reported as frequent findings in heartworm dogs (Nelson & Couto, 2015), is rare. Anemia, on the other hand, observed in 11.8% (4/34) of the dogs, may have been related to the trauma that the movement of the parasites causes to the red blood cells and vascular walls (Madril et al., 2020).

Because all infections were natural, it is impossible to infer how long these animals were infected, particularly because the course of the disease can be affected by the patient's physical activity, host immune response, parasitic load, and time of infection (Nelson et al., 2014). Nevertheless, no patient showed any signs of severe disease, suggesting that whatever the reason for not presenting severe signs, their infections could have been underdiagnosed in the absence of screening tests.

One of the red flags that raise the importance of testing is the finding of microfilariae on whole blood smears prepared for CBC. Only 25.8% (8/31) of the microfilaremic dogs presented microfilariae on these smears, confirming that this low sensitivity should be interpreted as an indication of the need for further investigation. However, Knott's test may miss some infections, as observed in this study. Three (8.8%) of the *D. immitis* antigen-positive dogs had no microfilariae detected by the Knott's test, which was much lower than the expected 30% of occult infections (Nelson et al., 2014).

In the preparation of microfilariae counting, only 20 smears of the 31 (64.5%) microfilaremic samples contained microfilariae. This reduced number in relation to the modified Knott's test detection suggests that when the larval load is low, 20 μ L may be insufficient to allow detection, as previously reported (Dillon, 2007; Nelson et al., 2014).

Conclusion

Veterinarians working in *D. immitis*-endemic areas should always undergo screening tests and pulmonary auscultation. Continuous expiratory lung sounds, even in the absence of coughing, must be interpreted as a sign of heartworm disease. CBC results vary among heartworm dogs, and eosinophilia, thrombocytopenia, and neutrophilia should include *D. immitis* infection in the differential diagnosis.

Ethics statement

The use of animals for this work was approved by the Ethics Committee on the Use of Animals (CEUA) of the Universidade Federal Fluminense under the number 499.

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None.

Conflict of interests

There is no conflicts of interest.

Authors' contributions

AJRB - Writing, Review and Editing manuscript. BA - Participate in microfilaria counting and review the manuscript. SG - The pathologist veterinarian responsible for blood analyses and review the manuscript. NL - Writing, Review and Editing manuscript. FMA - project coordinator and review the manuscript.

Availability of complementary results

The manuscript refers to a scientific article, with no research data available online.

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