How does *Dirofilaria immitis* infection impact the health of dogs referred to cardiology care

Como a infecção por *Dirofilaria immitis* afeta a saúde de cães encaminhados para atendimento cardiológico

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Abstract

Dirofilaria immitis is a nematode that can cause a disease that may present clinical signs from severe to absent. When dogs are symptomatic, the clinical signs are cardiorespiratory and nonspecific, which may be misleading. This study aimed to demonstrate the clinical presentations to cardiac care by evaluating 26 dogs subjected to clinical examination, complete blood count (CBC), specific tests for *D. immitis* infection, chest radiography, and echocardiography. Among them, 11 (42.3%) dogs were infected and 15 (57.7%) were non-infected. Most dogs presented with coughing (65.4%) and abnormal lung sounds (81%) independent of infection. Murmur at the tricuspid focus was present in 26.9% of the dogs, of which 57.1% were infected. Echocardiography revealed tricuspid regurgitation in 30.8% of the dogs and pulmonary regurgitation in 46.1%, of which 37.5% and 50% were infected, respectively. Worms were detected by echocardiography in 45.5% of the infected dogs. The x-rays showed that the bronchial pattern was present in 45.5% of the infected dogs and in 46.7% of the non-infected dogs. The interstitial pattern was present in 18.2% of the infected animals, in contrast to 6.7% of the non-infected dogs. The CBC results for all dogs were within the reference range, except for platelets. Although similar, the percentage of dogs with thrombocytopenia was higher among infected dogs (36.4%) than among the non-infected (6.7%). These results reinforce that due to the non-specific signs of infection, it is mandatory to perform parasitological assays when evaluating dogs presenting with cardiopulmonary signs.

Keywords: canine heartworm, clinical signs, dogs, cough.

Resumo

Dirofilaria immitis, é um nematoide que pode causar sinais clínicos de graves a ausentes. Quando os cães são sintomáticos, os sinais clínicos são cardiorrespiratórios e inespecíficos, o que pode confundir com outras doenças. Com o objetivo de descrever a apresentação clínica de cães atendidos em um serviço de cardiologia e doenças respiratórias, após consentimento dos tutores, 26 pacientes foram submetidos a exame clínico, hemograma, exames específicos para infecção por *D. immitis*, radiografia de tórax e ecocardiografia. Entre esses cães, 11 estavam infectados (42,3%) e 15 não infectados (57,7%). A maioria dos cães apresentou tosse (65,4%) e sons pulmonares anormais (81%) independentemente da infecção. O sopro no foco tricúspide estava presente em 26,9% dos cães, sendo 57,1% infectados. A ecocardiografia mostrou insuficiência tricúspide em 30,8% dos cães e insuficiência pulmonar em 46,1%, sendo 37,5% e 50% infectados, respectivamente. Parasitos foram detectados pela ecocardiografia em 45,5% dos cães infectados. As radiografias mostraram que o padrão brônquico estava presente em 45,5% dos cães infectados e em 46,7% dos não infectados. O padrão intersticial esteve presente em 18,2% dos infectados e em 6,7% dos não infectados. Os resultados do hemograma de todos os cães estavam dentro do intervalo de referência para todas as células, exceto plaquetas. Embora semelhante, a porcentagem de cães com trombocitopenia foi maior entre os cães infectados (36,4%), do que entre os livres de infecção (6,7%). Esses resultados reforçam que, devido aos sinais inespecíficos da infecção, é obrigatória a realização de ensaios parasitológicos na avaliação de cães que apresentem sinais cardiopulmonares.

Palavras-chave: doença do verme do coração, sinais clínicos, cães, tosse.

Introduction

Canine heartworm disease is caused by the nematode, *Dirofilaria immitis*, with the adult worms mainly inhabiting pulmonary arteries (American Heartworm Society, 2020; Knight, 1987; Venco, 2005). Although many *D. immitis*-infected dogs present no clinical signs (American Heartworm Society, 2020; Guerrero, 2005; European Society of Dirofilariosis and Angiostrongylosis, 2017), if left untreated, they may develop nonspecific and varied cardiorespiratory complications (American Heartworm Society, 2020) that can be misdiagnosed and confused with other cardiorespiratory diseases (Keene et al., 2019; Maggiore, 2014; Montoya-Alonso, 2007).

Different complementary examinations can be performed to differentiate the cause of clinical manifestations, such as cough, exercise intolerance, and dyspnea, which are common in different cardiorespiratory or systemic diseases (Maggiore, 2014; Montoya-Alonso, 2007).

A complete blood count can confirm a diagnosis of anemia and other diseases that may justify the history of tiredness (Calvert & Rawlings, 1983; Polizopoulou et al., 2000).

Chest radiography allows the identification of abnormalities in the lung parenchyma, trachea, main bronchi, or even intrathoracic structures not related to the lungs, which can cause coughing, dyspnea, tiredness, and cyanosis. Chest radiography can also detect heart silhouette changes, which may suggest the presence of heart disease (Thrall, 2013). Echocardiography is the gold standard for diagnosing morphofunctional cardiac abnormalities (Boon, 2011). Heart disease is a common cause of cough and tiredness (Tilley & Goodwin, 2002). Although echocardiography can identify the presence of *D. immitis* in the pulmonary artery or right heart chambers, it has low sensitivity for the diagnosis of infection (Venco et al., 2017). Therefore, antigen and microfilariae tests are considered the standard diagnostic aids of infection (American Heartworm Society, 2020).

Since asymptomatic canine heartworm disease is frequent and when clinical signs are present, they are similar to those of other etiologies, it may be difficult for the attending veterinarian to include *D. immitis* in the differential diagnostic work. The aim of the present study was to analyze the clinical signs presented by the dogs presented to the cardiorespiratory care unit of the Hospital Veterinário de Pequenos Animais (HVPA) of the Universidade Federal Rural do Rio de Janeiro (UFRRJ) to show to the practitioners how misleading the cardiorespiratory clinical signs may be.

Material and methods

The study was carried out at the Serviço de Cardiologia e Doenças respiratórias (SCDR) of Hospital veterinário da Universidade Federal Rural do Rio de Janeiro, with support from the Laboratório de Patologia Clínica Veterinária (LABVET), from October 2019 to February 2020.

Animals

The inclusion criteria for the enrollment of dogs were as follows: i) owners should sign the consent form and ii) dogs should be over 12 months of age. Exclusion criteria were i) brachycephalic dogs, ii) dogs presenting with congestive heart failure due to non-heartworm diseases, and iii) any missing examinations.

All information was recorded in an individual data capture form. The animals were subjected to a history evaluation, physical examination, complete blood count (CBC), *D. immitis* microfilariae and antigen tests, echocardiography, and chest radiography. All *D. immitis* tests were performed by the same technologist and echocardiography or chest radiography was performed by the same blinded veterinarian at all times.

History of the dogs' health, with special attention to coughing, dyspnea, exercise intolerance, or syncope, was recorded. The dogs were then examined and cardiopulmonary evaluation was carefully conducted.

Blood samples

Blood samples were collected in two EDTA tubes; one was used to perform modified Knott's test (Newton & Wright, 1956) and the specific *D. immitis* antigen test by ELISA (SNAP 4DX PLUS® IDEXX). The other tube was used for complete blood count, which was performed using a fully automated analyzer (Poch-100 iV; Sysmex/Roche, USA) in accordance with the manufacturer's recommendations. The hematological parameters obtained included total erythrocyte, leukocyte,

and platelet counts; hemoglobin concentration; packed cell volume; mean corpuscular volume; and mean corpuscular hemoglobin concentration. Differential leukocyte counts and cytological examinations were performed manually on Diff Quick-stained (Laborclin, Brazil) thin blood films using an optical microscope with immersion objective lens magnification of 1000x (Jain, 1993). Total plasma protein concentration was determined using refractometry.

Cardiopulmonary assessment

Thoracic radiographs were obtained using an X-ray emitter (Intercal® model Cr125-300mA) with image capture using digital processing (Carestream® CR Vita Flex), and were taken in the right, left, and ventrodorsal positions. Through radiography, the pulmonary pattern, pulmonary trunk, caudal lobar pulmonary artery, and cardiac silhouette were evaluated by the same blinded veterinarian, according to Thrall (2013).

Doppler echocardiography was performed using an Esaote MyLab Gamma® device with multifrequency sectoral transducers (1–4 MHz or 4–11 MHz). Left ventricular function as well as the morphology and function of the heart valves were evaluated according to Madron (2016) to identify changes that could affect the function of the right ventricle.

The probability of pulmonary arterial hypertension was evaluated according to the recommendations of the American College of Veterinary Internal Medicine (Reinero et al., 2020).

Right ventricular (RV) systolic function was assessed using the fractional change in RV area (FAC), tricuspid annular plane systolic excursion (TAPSE), and measurement of S-wave velocity. Animals with decreased values were considered to have systolic dysfunction (Madron, 2016).

RV diastolic function was assessed based on transtricuspid flow, the relationship between E and A waves, and tissue Doppler of the RV (E'/A'). Animals that presented at least one of the variables with decreased values were considered to have diastolic dysfunction. The ratio of E/A waves in the transtricuspid flow and E'/A' waves were considered normal when the values were above 1.12 (Madron, 2016).

The right cardiac chambers and pulmonary arteries were evaluated for linear structures of approximately 1.3 mm in diameter that may be considered as *D. immitis* adult worms (Venco et al, 2017).

Statistical analysis

For quantitative variables, measures of central tendency (mean, median, and mode) and variation (standard deviation, variance, and coefficient of variation) were calculated, and for qualitative variables, absolute and relative frequencies were estimated. Comparisons between the non-parametric means were performed using the Mann Whitney test, and the comparison between frequencies was performed using the Chi-square or Fisher's exact test depending on the value of n. All analyses were performed using the SPSS 25.0 program (IBM), and a 5% probability was considered.

Results

Animals

Among the 31 dogs initially included in the study, four were excluded due to missing exams, and one was brachycephalic. Of the 26 dogs included, 11 (42.3%) harbored heartworms and 15 (57.7%) were infection-free. Among the infected dogs, five (45.4%) presented with microfilariae and antigens, four (36.4%) presented occult infections, and two (18.2%) presented only microfilariae. Microfilariae were identified as *D. immitis* using the Knott test (American Heartworm Society, 2020).

Clinical assessment

There was no difference between the infected and non-infected dogs when compared to the variables evaluated in the clinical examination (Table 1). Ascites was not detected in any of the studied animals. The owners denied syncope for all animals. Notably, the owners correctly reported coughing (65.4%, 17/26) when compared with the positive tracheal coughing reflex detected by the veterinarians (80.8%, 21/26).

Table 1. *Clinical assessment of Dirofilaria immitis infected dogs (HTW+) and Dirofilaria immitis infection free dogs (HTW-) presented to cardiology care of veterinary hospital of Universidade Federal Rural do Rio de Janeiro according to clinical assessment.*

	HTW +		HTW –		P-Value
	N	n (%)	N	n (%)	
Positive Tracheal Coughing Reflex	11	7 (63.6)	15	14 (93.3)	0.082
Exercise intolerance	11	3 (27.3)	15	4 (26.7)	0.655
Tricuspid Heart Murmur	11	4 (36.4)	15	3 (20)	0.313
Pulmonary Heart Murmur	11	1 (9.1)	15	0 (-)	0.423
Lung Auscultation Abnormalities	11	9 (81.8)	15	12 (80)	0.654

Blood samples

The hematological values of infected and non-infected dogs were similar and within the normal range for all cells except platelets. Although similar, the percentage of dogs with thrombocytopenia was higher among the infected dogs (36.4%, 4/11) than among those free from infection (6.7%, 1/15) (X^2=1.94; p =0.1279).

Cardiac evaluation

All dogs evaluated in the study, whether infected or uninfected, had at least one cardiac abnormality. When evaluating the chest radiograph in ventrodorsal position in a subjective and clock-like manner, right atrioventricular enlargement was suggested in 38.5% (10/26) of the animals. This could not be associated with heartworm infection because its frequency was even higher in the uninfected group (60%, 6/10) (Table 2).

Tricuspid regurgitation was observed in 30.8% (8/26) and pulmonary regurgitation was observed in 45.1% (12/26) of the dogs. Although not significantly different, tricuspid regurgitation was more frequent among the HTW-free dogs (33.3%, 5/15), and pulmonary regurgitation was higher among the HTW-infected dogs (54.5%, 6/11) (Table 2). Right ventricular systolic dysfunction was identified in 34.6% (9/26) of the dogs, of which 44.4% (4/9) were infected. The analysis of diastolic dysfunction was similar between infected and non-infected dogs; However, interestingly, among the infected dogs, 54.5% (6/11) had alterations on tissue Doppler and among the non-infected, 26.7% (4/15) showed alterations in transtricuspid flow (Table 2).

Table 2. Cardiac evaluation of *Dirofilaria immitis* infected dogs (HTW+) and *Dirofilaria immitis* infection free dogs (HTW-) presented to cardiology care of veterinary hospital of Universidade Federal Rural do Rio de Janeiro according to cardiac evaluation.

	HTW +		HTW –		P-value
	N	n (%)	N	n (%)	
Right atrioventricular enlargement*	11	4 (36.3)	15	6 (40)	0.9706
Tricuspid regurgitation**	11	3 (27.3)	15	5 (33.3)	0.9530
Pulmonary regurgitation**	11	6 (54.5)	15	6 (40)	0.9747
S wave abnormalities**	11	3 (27.3)	15	4 (26.7)	0.4793
TAPSE Decrease**	11	3 (27.3)	15	9 (60)	0.0806
FAC Increase**	11	8 (72.7)	15	6 (40)	0.0806
E/A Decrease**	11	1 (9.1)	15	4 (26.7)	0.2259
E'/A' Decrease**	11	6 (54.5)	15	12 (80)	0.1065

Note. TAPSE: tricuspid annular plane systolic excursion; FAC: fractional change in the right ventricular area; E/A- transtricupid flow E'/A' – Tissue Doppler from the right ventricle. *Chest x-ray; **Echodopplercardiogram.

Pulmonary evaluation

All infected dogs presented with at least one lung abnormality, and among the 15 non-infected dogs, only two showed no pulmonary alterations (Table 3).

When comparing the different pulmonary alterations, there were no significant differences between the two groups of dogs. Pulmonary pattern abnormalities were the most frequent alteration (88.5%, 23/26). The bronchial pattern showed a similar frequency among the infected (45.5%, 5/11) and non-infected dogs (46.7%, 7/15). The interstitial pattern was (18.2%, 2/11) in the infected group and (6.7%, 1/15) in the HTW free groups, respectively. Bronchial and interstitial patterns were identified concomitantly in 27.3% (3/11) of infected and 33.3% (5/15) of non-infected dogs. No alveolar or micronodular patterns were observed. Adult worms could only be detected in 5.5% (6/11) of the heartworm infected dogs (5.5% - 6/11).

Table 3. Pulmonary evaluation of *Dirofilaria immitis infected dogs (HTW+) and Dirofilaria immitis infection free dogs (HTW-)* presented to cardiology care of veterinary hospital of Universidade Federal Rural do Rio de Janeiro according to lungs evaluation.

	HTW +		HTW –		P-value
	N	n (%)	N	n (%)	
Main Pulmonary Artery Enlargement *	11	3 (27.2)	15	4 (26.7)	0.9855
Enlargement of the caudal lobar pulmonary artery*	11	2 (18.2)	15	3 (20)	0.9566
Pulmonary Pattern Abnormalities*	11	10 (90.9)	15	13 (86.7)	0.4484
HTW **	11	5 (45.5)	15	0	0.9748
AP/AO Increase **	11	10 (90.9)	15	11 (73.3)	0.2259
IDAP decrease**	11	1 (9.1)	15	1 (6.7)	0.4587

Note. N, number of animals; AP/AO, aorta pulmonary artery ratio; IDAP, pulmonary artery distensibility index. *Chest x-ray; **Echodopplercardiogram.

Discussion

The percentage of dogs presenting with cough reported by the owners among the number of positive cough tracheal reflexes detected by the veterinarians (80.95%, 17/21) suggests that the attending veterinarians must value the information provided by the owner. On the other hand, subjective signs, such as intolerance to exercise, may not to be evident to the owners because they are insidious. Less than 30% of the owners of infected (27.3%, 3/11) or non-infected (26.7%, 4/15) dogs mentioned exercise intolerance, although the pulmonary pattern abnormalities detected in most dogs (88.5%–23/26) suggest that more dogs should present it, according to previous reports (Calvert & Thomason, 2008; Feitosa, 2020; Gompf, 2008).

Coughing should not be solely considered as an indication of HTW disease once, despite being frequent in these dogs (McHaffie, 2012; Simón et al., 2012) as non-infected positive cough tracheal reflex dogs (90.3%–14/15) were more frequent than coughing among the infected (63.6%, 7/11).

The absence of syncope or ascites, as well as the few reports of dyspnea, show that most animals, although sick, are still in the initial stages of cardiorespiratory diseases (Calvert & Thomason, 2008; Gompf, 2008).

Although physical examination showed that the changes detectable on cardiac auscultation could not be associated with infection, abnormalities such as heart murmur are a common finding in most heart diseases (Tilley & Goodwin, 2002). Likewise, although the majority of dogs presented abnormalities in pulmonary auscultation (81%), they could not be associated with infection. However, alterations in the evaluation of the lungs on clinical assessment can be indirectly observed by most owners in the form of cough (65.4%). However, it is worth mentioning that coughing of extrapulmonary origin can occur (Keene et al., 2019; Maggiore, 2014) and influence the diagnosis. Therefore, coughing should be considered cautiously because it can occur in the absence of pulmonary changes (Gompf, 2008).

Evaluation of the cardiac silhouette in the radiographic examination did not suggest Cor pulmonale in most animals, regardless of the infection. Right atrioventricular enlargement was observed in fewer infected animals (36.4%) than expected, as it is a frequent finding in heartworm disease (American Heartworm Society, 2020; Calvert & Rawlings, 2002; McCall et al., 2008). Therefore, it may be suggested that an increased pulmonary arterial pressure capable of cardiac remodeling (Jessup et al., 1987; MacNee, 1994; Render et al., 1995; Simón et al., 2012) is absent in most animals. However, enlargement of the right heart chambers identified on radiography was frequent in non-infected dogs (40%), suggesting that other causes of Cor pulmonale occurred because these patients were treated at SCDR with a suspected disease that may present with right atrial enlargement. Therefore, radiographic evaluation may overestimate the heart size depending on patient positioning (Thrall, 2013).

Interestingly, most of the animals presenting with diastolic dysfunction were over six years old. This may have been influenced by the fact that elderly dogs have a lower relaxation capacity in the ventricular myocardium (Boon, 2011) even though diastolic dysfunction has been reported to be frequent in naturally infected dogs, regardless of age (Alberigi et al., 2020).

When myocardial dysfunction of the RV of the infected dogs is considered, it may be observed that all dogs presenting with systolic dysfunction also presented diastolic dysfunction, which may indicate that in *D. immitis* infection, diastolic dysfunction occurs before systolic dysfunction, similar to other conditions (Nishimura & Tajik, 1997).

Although the high percentage of bronchial and interstitial patterns in infected dogs can be explained by the inflammation resulting from the lesions caused by the worms and their endosymbionts (Bandi et al., 2001; Labarthe et al., 2009; Rawlings, 1986; Simón et al., 2012); the high percentage of those patterns among the non-infected dogs was not surprising, as most of the dogs referred to the cardiology department presented cardiorespiratory signs despite the infection. As non-infected animals presented a set of signs that suggested the presence of pulmonary hypertension on echocardiography, it can be inferred that the endothelial damage of the disease (Labarthe et al., 2009; Simón et al., 2012) was insufficient to compromise the pulmonary vasculature in the study dogs.

Although specific, the detection of worms by echocardiography presented low sensitivity (45.5%), confirming that even when the operator is experienced, the diagnosis should always include microfilariae and antigen tests (Venco et al., 2003).

No significant differences in hematological values between infected and non-infected dogs were detected, and all values were within the reference range for the species (Calvert & Rawlings, 1983). However, as previously reported, microfilaremic dogs may present with anemia, lymphocytosis, eosinophilia, or thrombocytopenia (Niwetpathomwat et al., 2007). The clinical stage of the disease presented by infected dogs was mild or moderate (American Heartworm Society, 2020), suggesting that the hematological changes, if present, were subtle.

Although the sample size was a limitation of this study, as well as the bias imposed by the group of dogs presented to the cardiac care unit, it is interesting to note that both infected and non-infected animals did not present severe disease. Clinical examination, CBC, and imaging examinations could not be considered accurate in determining parasitic etiology, even though echocardiography was able to partially detect infection. Therefore, the etiology requires specific investigation of microfilariae and specific antigen detection.

Conclusions

Dogs referred for cardiology care presented similar clinical and complementary examination findings, regardless of whether they were infected with *D. immitis*. Therefore, thorough clinical examination and specific parasitological tests must be performed to rule out infection.

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Ethical considerations

This study was approved by the Animal Use Ethics Committee of the Instituto de Veterinparia of the Universidade Federal Rural do Rio de Janeiro (with protocol number: 87658445720).

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Conflicts of interest

NMOL, BA, FBK, CDB, MFAS – No conflict of interest. NL is a consultant for Boehringer Ingelheim, IDEXX, and Zoetis in Brazil. JL is a consultant and research collaborator with IDEXX and Zoetis.

Authors' contributions

NMOL, BA, NL, MFAS – Contributed of idealization of the study. NMOL - Contributed of assisted the reported patients. BA - Contributed of echocardiogram performance. FBK - Contributed of application of statistical study data. NMOL, BA, NL, FBK, CDB, MFAS - contributed to the writing and review of the manuscript

Availability of complementary results

All information obtained as a result of the study is included in the manuscript

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