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Cardiopulmonary radiographic changes in dogs naturally infected with *Dirofilaria immitis*[1]

Alexandre M. Ferreira[2], Carlos Eduardo C. Netto[2], Carlos Augusto Santos-Sousa[3], Paulo Souza Júnior[4], Hélio José S. Bagetti Filho[5] and Marcelo Abidu-Figueiredo[5]*

ABSTRACT.- Ferreira A.M., Netto C.E.C., Santos-Sousa C.A., Souza Júnior P., Bagetti Filho H.J.S. & Abidu- Figueiredo M. 2024. **Cardiopulmonary radiographic changes in dogs naturally infected with *Dirofilaria immitis*.** *Pesquisa Veterinária Brasileira 44:07466, 2024.* Departamento de Anatomia Animal e Humana, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Rodovia BR-465 Km 7, Seropédica, RJ 23897-000, Brazil. E-mail: marceloabidu@gmail.com

Heartworm disease is a zoonosis caused by *Dirofilaria immitis*, a nematode parasite of worldwide distribution and considered one of the most virulent of dogs, which are definitive and infected hosts during the blood meal of vector mosquitoes (*Aedes* sp., *Anopheles* sp. and *Culex* sp.). The disease has a worldwide prevalence, with a higher incidence in tropical and subtropical climate areas, and affects animals of any breed, sex, and age group. Most animals are asymptomatic, but the disease can cause various cardiovascular and respiratory symptoms. The pathophysiology is related to vessel obstruction and an intense inflammatory reaction in the lung parenchyma, with cardiovascular and respiratory symptoms leading to signs that can be severe and lead to the death of the host. It can be diagnosed through blood tests, and chest X-rays are used to assess the severity of clinical signs and help establish a prognosis. Cardiovascular alterations appear as an increase in vascular caliber, an increase in the right heart silhouette, and pulmonary patterns compatible with infection. This study aimed to describe the conditional radiographic changes caused by the parasite in the lungs, intrathoracic vessels and heart in 30 dogs, independent of breed and sex, with an average age of 9.9 years and an average weight of 10.25kg, originating from routine care at a veterinary hospital in the city of Rio de Janeiro, from January 2022 to December 2022. All animals were diagnosed with a positive heartworm infection and cardiorespiratory symptoms as essential conditions for participation in the study. The animals were x-rayed and had their lung patterns evaluated, in addition to measuring their vascular caliber and cardiac indices using vertebral-heart size (VHS), manubrium-heart size (MHS) and sphericity index (CSI). Most animals presented pulmonary patterns such as bronchoalveolar and bronchial and increased VHS and MHS indices. Increased and borderline values of CSI measurements were found in half of the patients, demonstrating that the parasite can trigger significant radiographic changes or present potential further worsening.

INDEX TERMS: *Dirofilaria immitis*, diagnostic imaging, radiographic changes.

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[2] Graduate Program in Clinical and Animal Reproduction, Universidade Federal Fluminense (UFF), Av. Alm. Ary Parreiras 507, Icaraí, Niterói, RJ 24230-321, Brazil.

[3] Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Rua da Glória 187, Centro, Diamantina, MG 39100-000, Brazil.

[4] Universidade Federal dos Pampas (Unipampa), Av. Maria Anunciação Gomes Godoy 1650, Bagé, RS 96460-000, Brazil.

[5] Professor, Departamento Anatomia Animal e Humana, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rodovia BR-465 Km 7, Seropédica, RJ 23897-000, Brazil. *Corresponding author: marceloabidu@gmail.com

RESUMO.- [**Alterações radiográficas cardiopulmonares em cães naturalmente infectados por *Dirofiliaria immitis*.**] A dirofilariose é uma zoonose causada pela *Dirofilaria immitis*, parasita nematoide de distribuição mundial e considerado um dos mais patogênicos de cães, que são hospedeiros definitivos e infectados durante o repasto sanguíneo de mosquitos vetores (*Aedes* sp., *Anopheles* sp. e *Culex* sp.). A doença apresenta prevalência mundial, com maior incidência em áreas de clima tropical e subtropical, acometendo animais de qualquer raça, sexo e faixa etária. A maioria dos animais é assintomática, entretanto, a doença pode causar diversos sintomas cardiovasculares e

respiratórios. A fisiopatologia se relaciona com a obstrução de vasos e com intensa reação inflamatória no tecido pulmonar, com sintomatologia cardiovascular e respiratória, levando a sinais que podem ser graves e levar ao óbito do hospedeiro. Pode ser diagnosticada através de exames de sangue e as radiografias de tórax são utilizadas para avaliar a gravidade dos sinais clínicos e auxiliar no estabelecimento do prognóstico. Alterações cardiovasculares surgem como aumento de calibre vascular, aumento de silhueta cardíaca direita e padrões pulmonares compatíveis com infecção. No presente artigo objetivou-se descrever as alterações radiográficas estabelecidas pelo parasita nos pulmões, nos vasos intratorácicos e no coração em 30 cães, independente de raça e sexo, com média de idade 9,9 anos e média de peso 10,25kg, oriundos da rotina de atendimento de um hospital veterinário na cidade do Rio de Janeiro, entre Janeiro de 2022 e Dezembro de 2022. Todos os animais possuem diagnóstico positivo para infecção por dirofilariose e sintomatologia cardiorrespiratória como condição indispensável para pertencerem ao estudo. Os animais foram radiografados e tiveram os padrões pulmonares avaliados, além da medição do calibre vascular e dos índices cardíacos através do VHS (índice cardio-vertebral), MHS (ou índice manúbrio-esternal) e CSI (índice de esfericidade). A maioria dos animais apresentou padrão pulmonar como broncoalveolar e bronquial e índices de VHS e MHS aumentados. Aumento e valores limítrofes das medidas de CSI foram encontrados em metade dos pacientes, demonstrando que o parasita pode desencadear alterações radiográficas significativas ou apresentar potencial agravamento posterior.

TERMOS DE INDEXAÇÃO: *Dirofilaria immitis*, diagnóstico por imagem, alterações radiográficas.

INTRODUCTION

Dirofilariasis is a disease caused by *Dirofilaria immitis* (Leidy, 1856), a nematode with worldwide distribution and considered one of the most pathogenic parasites of dogs, popularly known as "heartworm disease," affecting dogs in tropical, subtropical, and temperate regions worldwide (Rath et al. 2014).

The parasite is transmitted by mosquitoes of different species, with mandatory passage through these intermediate hosts, which belong to the genera *Aedes*, *Anopheles*, and *Culex* (Fernandes et al. 2000). Mosquitoes are dipteran insects belonging to the family Culicidae, whose adults are winged and the females are hematophagous, with the blood meal related to the development of eggs (Rotraut & Oliveira 1994).

Filariae require a hematophagous arthropod (mosquito) to develop their life cycle (Silva & LangoniI 2009). Mosquito females are hematophagous because they need blood for the maturation of their eggs (Freitas et al. 1982), and they are viviparous, incubating the eggs in the uterus and releasing first-stage larvae (L1) into the circulating blood (Silva & LangoniI 2009). The cycle is relatively long, ranging from six to nine months. When a female mosquito feeds on a host infected with *D. immitis*, it may ingest microfilariae in the bloodstream. These ingested microfilariae (L1 larvae) change to the L2 larval stage ten days after infection. About thirteen days after mosquito infection, the L3 larval stage emerges. The L3 larvae, already sexually differentiated, penetrate the host through the wound caused by the mosquito bite. They molt to L4 three to four days after infecting the definitive host and to L5 after seventy or more days. The adults become sexually mature three months after reaching the right ventricle via the venous circulation. If there are adult parasites of both sexes, microfilariae are produced that circulate in the bloodstream (Meireles et al. 2014).

The diagnosis is based on the detection of microfilariae in the circulation (Magnis et al. 2013). Chest radiography plays an important role in evaluating the cardiovascular system, and diagnosing cardiac alteration includes assessing the size and shape of the silhouette through objective and subjective measures (Puccinelli et al. 2021). Chest radiography does not detect the parasite but is useful for detecting the disease caused by it, assessing the severity, and evaluating cardiopulmonary changes (Rath et al. 2014).

The increased permeability of vascular surfaces leads to perivascular edema, observed on chest radiography as an alveolar pattern. There is also an interstitial pattern due to vasculopathy, perivascular fluid extravasation, and inflammation (Ettinger & Feldman 2000). Edema in the peribronchovascular interstitium may appear as a bronchial/bronchiolar-interstitial pattern.

Chest radiography to evaluate cardiac size is also important for the clinical management of cardiovascular disease by quantifying cardiac chambers and providing a non-invasive estimate of disease severity (Duler et al. 2021).

The diagnosis of cardiac changes is not based solely on radiographic findings, requiring the association of clinical signs and electro- and echocardiographic examinations, suggesting the prognosis and treatment to monitor the disease's progression. Several studies have been conducted with the aim of increasing the accuracy and decreasing the subjectivity of the radiographic study of the silhouette, including evaluation through the vertebral-heart size (VHS) (Cardoso et al. 2011), the manubrium-heart size (MHS), and the sphericity index (CSI), in addition to the evaluation of lung patterns and vascular caliber.

This study aimed to demonstrate the cardio-vascular radiographic changes in dogs infected with *Dirofilaria immitis*, identifying lung patterns, measuring the VHS, MHS, CSI indices, and the caliber of the caudal vena cava.

MATERIALS AND METHODS

Animal Ethics. This project was approved by the Ethics Committee on Animal Use (CEUA) of the "Universidade Federal Fluminense" (UFF), under protocol number 1243100923.

Study site and contextualization. Thirty adult dogs of independent breed and sex were used, with an average weight of 10.2kg and an average age of 9.9 years. The animals come from the routine care of a veterinary hospital located in the city of Rio de Janeiro.

Clinical data. All animals in the study have a positive diagnosis for dirofilariasis with cardio-respiratory symptoms such as cough, dyspnea, fatigue, and apathy, regardless of breed and sex, with an average age of 9.9 years and an average weight of 10.25kg, and underwent chest radiographic examination. Brachycephalic breeds and animals under one year of age were excluded from the study. All animals presented a Free and Informed Consent Form submitted to their respective guardians.

Sample acquisition. The animals were physically restrained and subjected to right lateral and ventrodorsal positioning. The imaging equipment used is owned by the hospital and consists of an x-ray emitter from Siemens, model Heliophorus 500mA, and a digitalizer from Agfa, model CR-30, with NX software. The acquired

images were sent to the Picture Archiving and Communication System (PACS) image file system, where they were analyzed on a high-resolution BARCO monitor. All measurements were made using the public domain software ImageJ (National Institutes of Health).

Vertebral-heart size (VHS). The VHS is the most common technique for evaluating the cardiac size of dogs in chest radiographs. For VHS measurement, the long axis of the silhouette is measured by drawing a line from the central and ventral edge to the carina region of the trachea up to the cardiac apex. A short axis is drawn from the widest part of the silhouette within the central third region in the dorsoventral plane perpendicular to the long axis. The measurements of the two axes are indexed to the thoracic vertebral bodies, starting at the cranial aspect of the T4 vertebra, and summed (Duler et al. 2021). The normal VHS value is set at 10.5 vertebral bodies in most breeds (Fig.1).

Manubrium-heart size (MHS). The MHS is an alternative to VHS in determining cardiac dimensions by evaluating a readily identifiable structure, the manubrium. The index determination in assessing the overall size of the silhouette is done by measuring each cardiac axis (short-cSAL and long-cLAL) in the right lateral and ventrodorsal projections (Mostafa & Berry 2017). In the ventrodorsal radiograph, the long axis is measured from the tracheal carina edge's ventral point to the apex's caudoventral margin. The short axis is measured at the widest point of the silhouette on a line perpendicular to the long axis. The length of the manubrium is measured in the right lateral projection (Mostafa & Berry 2017). According to Capuzzi (2020), the MHS is evaluated by adding the short and long axes and dividing by the length of the manubrium. Although they can be used as reference values, further studies are needed to substantiate the data found in MHS evaluation (Fig.2 and 3).

Sphericity index (CSI). The sphericity index can be used to assess the overall size of the cardiac chambers. An index greater than or equal to 1.0 indicates an almost round silhouette shape, indicating changes in the organ. It is obtained by the ratio between the short and long axes measured in VHS, with normal values ranging from 0.69 to 0.92. The sphericity index is considered a complementary parameter to assess cardiac size in cases where VHS cannot be used, consisting of an important parameter in cardiomyopathies involving the right and left sides. However, it is less relevant in cases of global cardiomegaly because the short and long axes increase proportionally. Right-sided cardiomegalies increase the sphericity index more than left-sided cardiomegalies, as the enlarged right side causes more sphericity (Bappah et al. 2021) (Fig.4).

Vascular caliber. Changes in the pulmonary vessels caused by the parasite can lead to inflammation and thrombosis, determining changes in their caliber, which can be attributed to pulmonary hypertension. The presence of parasites in the pulmonary arteries causes proliferative endarteritis, arterial lumen narrowing, reduced distensibility, and arterial embolism, resulting in increased pulmonary pressure (Romano et al. 2021). The parameters evaluated are the ratio between the caudal vena cava and the aorta (CdVc/Ao) and the ratio between the caudal vena cava and the length of the T4 vertebra (CdVc/VL). High proportions of the caudal vena cava diameter relative to the length of T4 and the diameter of the aorta suggest right heart disease in dogs infected with *Dirofilaria immitis*. CdVc/Ao greater than 1.5 and CdVc/VL greater than 1.30 suggest right heart changes (Pajas & Acorda 2018) (Fig.5).

Pulmonary patterns. For the evaluation of the lung fields, we followed the description by Thrall (2019), where pulmonary radiographic abnormalities are classified according to the involvement of the alveoli, bronchi, or interstitium.

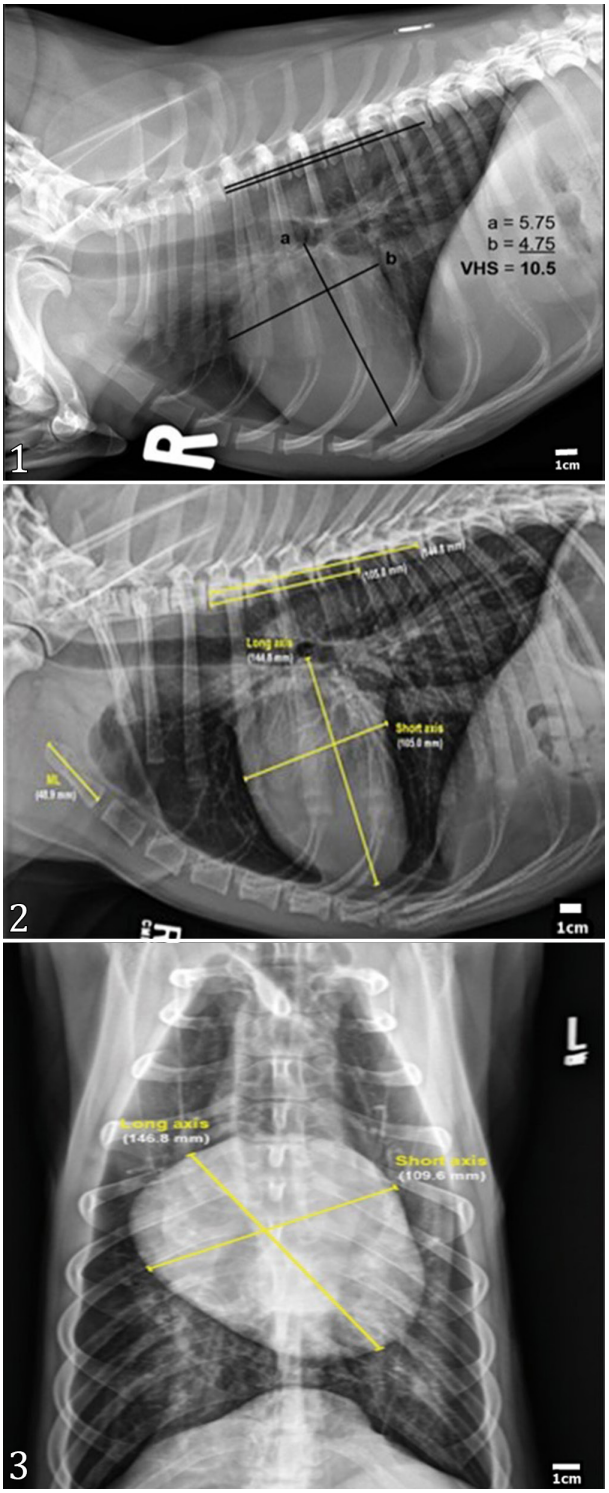

Fig.1-3. (**1**) Right lateral radiographic image illustrating the reference points for vertebral-heart size (VHS) measurement. Adapted from Birks et al. (2017). (**2**) Right lateral radiographic image illustrating measurements along the spine used for VHS determination and measurements of manubrium length (ML), cardiac short-axis length (cSAL), and cardiac long-axis length (cLAL) used for total manubrium-heart size (MHS) determination. Adapted from Mostafa & Berry (2017). (**3**) Ventrodorsal radiograph of a dog, illustrating the measurements of ML, cSAL, and cLAL for total MHS determination. Adapted from Mostafa & Berry (2017). Bar = 1cm.

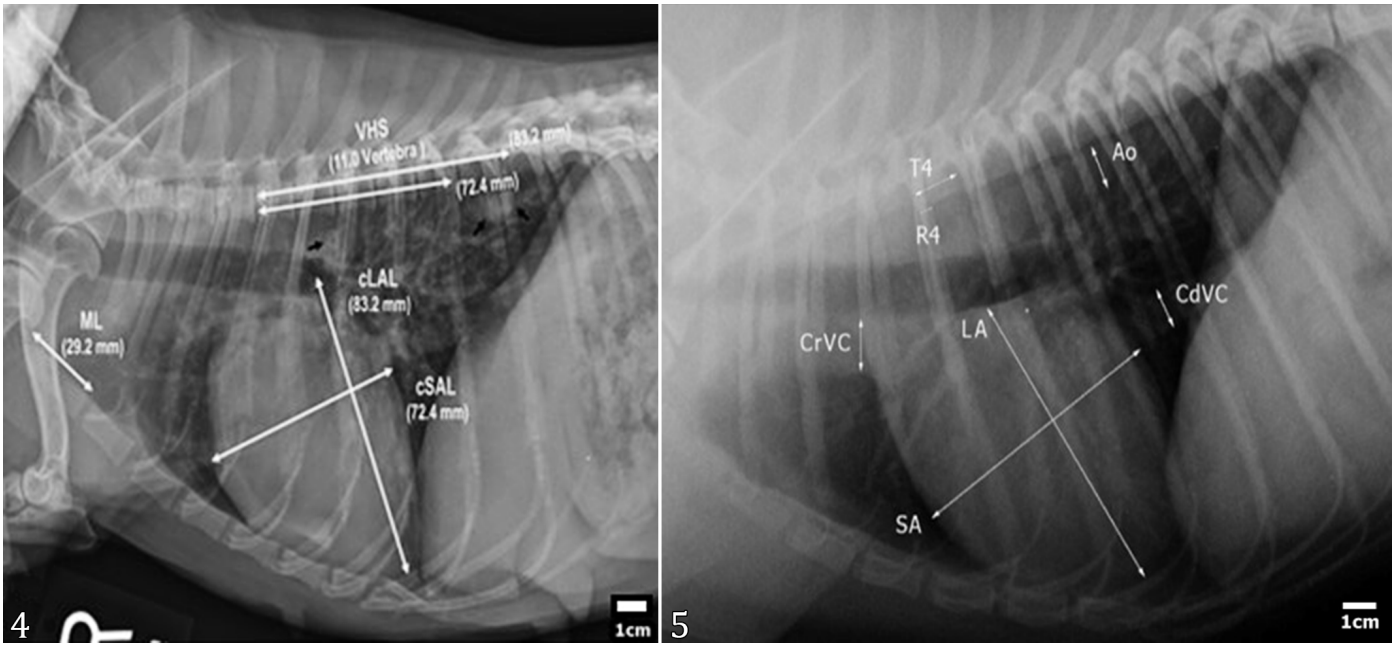

Fig.4-5. (**4**) Right lateral radiograph of a dog with dirofilariasis and right-sided cardiomegaly showing measurements for cardiac long-axis length (cLAL), cardiac short-axis length (cSAL), and manubrium length (ML) and the results for sphericity index (CSI), vertebral-heart size (VHS), and short, long, and total manubrium-heart size (MHS): cLAL = 83.2mm, cSAL = 72.4mm, ML = 29.2mm, CSI = 0.87 or 87%, global MHS = 5.4, VHS = 11.0. Adapted from Mostafa et al. (2020). (**5**) Right lateral incidence radiograph demonstrating measurements of the short and long axes, as well as the caliber of the caudal vena cava and aorta, T4 length, and fourth rib width. Bar = 1cm.

RESULTS

The 30 animals used in this study were properly identified and presented by breed, sex, weight, and age, and their VHS, MHS, and CSI indices were calculated (Table 1).

Pulmonary patterns

Bronchoalveolar and bronchial patterns were evaluated in 33% of cases, alveolar in 18%, and interstitial alveolar in 16%, indicating changes in lung tissue due to the inflammatory process caused by the parasites.

Measurements of VHS, MHS, and CSI

In 73% of the cases analyzed, there was an increase in VHS values, and in 70% of cases, there was an increase in MHS values. CSI values were increased in 10% of cases; however, the values were borderline in 47% of the animals analyzed. The increased values indicate a change in the size of the cardiac silhouette, demonstrating that the parasites can determine morphological changes due to their effects. Borderline values indicate a possible further worsening of the clinical condition.

Vascular caliber measurements

The relations between the caudal cava with the aorta and the length of T4 were increased by 25% and 9%, respectively, but 7% and 9% were at borderline values. The increased values indicate a change in vascular caliber, demonstrating that the parasites can cause changes at the endothelial level, leading to luminal thickening with resistance to flow and, consequently, hypertension. Borderline values indicate a possible further worsening of the clinical condition.

DISCUSSION

The radiographic changes in the thorax are described based on the increase or decrease in radiopacity. Increased radiopacity is classified into patterns. Pulmonary patterns refer to the radiographic changes presented by animals with respiratory symptoms. The patterns found in the animals of this study were compatible with bronchopneumopathies, with the majority being bronchial and bronchoalveolar patterns (both 33%), as the presence of parasites causes an inflammatory reaction resulting in the bronchial pattern and increased vascular permeability resulting in the alveolar pattern. These patterns can occur alone or together, depending on the severity of the clinical condition. Some patients presented with the alveolar pattern alone (18%), indicating increased vascular permeability without bronchial changes, while others (16%) presented with an interstitial pattern, indicating changes at the level of pulmonary interstitium (connective tissue), suggesting that infection with *Dirofilaria immitis* can cause changes in pulmonary parenchyma that are visualized radiographically (Fig.1).

The VHS measurements showed an increase independent of breed in 73% of cases due to secondary changes caused by the parasites, indicating a higher prevalence of cardiomegaly secondary to *D. immitis* infection. Cardiomegaly occurs due to parasites in the pulmonary vessels and eventually in the cardiac chambers, generating resistance to blood flow and consequent hypertrophy, increasing the VHS. Borderline values were found in 10% of cases. In animals that did not show an increase in VHS, it is believed that the condition is not severe or that cardiac dimension changes have not yet occurred in early cases (Fig.2).

The increases observed in the long and short manubriosternal axes indicate an increase in the cardiac silhouette in 83% of cases, as these axes are evaluated by measuring from the ventral region at the carina of the trachea to the cardiac apex (long axis) and from the cranial cardiac waist measurement to the ventral aspect of the caudal vena cava (short axis), assessing the cardiac silhouette as a whole. With the increase in the long and short axes, there is consequently an increase in the total MHS, demonstrating an increase in the cardiac silhouette secondary to infection. Borderline values were found in 7% of cases. Similarly to VHS measurements, in animals that did not show an increase in MHS, it is believed that the condition is not severe or that they are in early stages (Fig.3). MHS measurements usually increase when there is an increase in VHS, as both use measurements of the long and short cardiac axes for their calculations and generally indicate a more severe clinical condition.

The cardiac silhouette normally has a more elongated radiographic shape. The shape can become more rounded or spherical when cellular changes occur in response to injuries. Changes in cardiac shape can be assessed through the CSI. In our study, the CSI showed changes in 3% of the animals; however, 87% showed borderline measurements, indicating the possibility of worsening infection later on. Mostafa et al. (2020) demonstrated that when the short and long axes increase proportionally, the ratio between them is not expected to change. Therefore, CSI should not be used solely to assess cardiac size but rather to obtain an estimate of cardiac shape. It is considered that MHS and CSI are higher in animals with confirmed heart disease. There were cardiorespiratory symptoms in the patients evaluated in this study, but there was no confirmation of isolated heart disease. Thus, heart disease may occur with the possibility of worsening conditions, and CSI may increase in response to infection. Patients with increased CSI, possibly in more severe cases with concomitant heart disease, are expected to have a change in the shape of the cardiac silhouette (Fig.4).

Our study demonstrated that vascular changes observed in the caudal vena cava led to inflammatory processes causing endarteritis, thromboembolism, and hypertension, resulting in an increase in its caliber in response to the difficulty in blood flow. Animals that presented with borderline vascular ratios (CdVc/Ao 7%, CdVc/VL 7%) may show the beginning of clinical worsening, and those that presented with an increased

Table 1. List of animals used and categorized by breed, sex, weight, and age, along with their respective indices (VHS, MHS, and CSI) obtained

Animal	Breed	Sex	Weight (Kg)	Age	VHS	MHS	CSI
1	MBD	F	4.5	7	11.0	5.9	0.9
2	MBD	M	18.0	4	11.0	6.0	1.0
3	Doberman	M	47.7	7	13.0	8.0	0.8
4	MBD	M	7.1	11	10.1	4.3	0.8
5	Shar-Pei	M	22.3	6	10.3	6.4	0.9
6	Poodle	F	7.0	4	11.0	4.7	0.8
7	Poodle	M	9.0	9	11.4	5.7	0.9
8	MBD	M	15.0	12	10.0	6.0	0.8
9	Rottweiler	F	47.0	6	10.3	4.7	0.8
10	Chow-Chow	M	20.0	6	12.7	5.5	0.9
11	MBD	M	8.2	12	11.8	5.3	0.7
12	Shih-Tzu	F	4.2	13	11.0	6.5	0.8
13	Rottweiler	M	47.0	6	11.7	5.6	0.8
14	MBD	F	10.0	4	12.0	9.3	0.9
15	MBD	M	11.2	15	12.0	4.9	0.8
16	Cocker Spaniel	F	15.0	8	13.0	5.2	0.8
17	Poodle	F	6.0	9	10.8	4.4	0.9
18	Labrador Retriever	M	15.0	4	9.5	4.2	0.9
19	MBD	M	10.0	8	10.5	5.6	0.7
20	German Shepherd	M	37.0	5	10.8	6.3	0.9
21	Yorkshire	F	4.0	10	10.8	6.2	0.9
22	Poodle	F	8.5	8	10.5	6.7	0.8
23	MBD	M	11.3	11	13.0	6.0	0.7
24	Rottweiler	F	43.0	6	10.5	5.0	0.9
25	MBD	M	5.2	8	11.3	6.1	0.8
26	Maltese	M	5.0	4	11.0	6.0	0.8
27	MBD	M	8.1	5	11.5	6.9	0.9
28	MBD	M	7.0	11	11.6	6.0	0.9
29	Cocker Spaniel	M	17.0	15	12.7	6.3	0.9
30	MBD	M	5.6	12	11.5	5.6	0.9

VHS = vertebral-heart size, MHS = manubrium-heart size, CSI = sphericity index, MBD = mixed breed dogs.

(CdVc/VL 10%) ratio may present possible caudal vena cava syndrome. On the other hand, patients who did not show alterations in the vascular ratios (CdVc/Ao 93% and CdVc/VL 83%) possibly do not have parasites in the location of the caudal vena cava (Fig.5).

CONCLUSIONS

Dirofilariasis is a disease that can produce severe symptoms, depending on the degree of infection. Common symptoms are cardiorespiratory with the possibility of developing thrombi. Although radiographic examination does not make the diagnosis, it identifies secondary changes by evaluating pulmonary patterns and measurements of the cardiac silhouette and vascular caliber.

Generally, the presence of parasites and their effects result in changes in pulmonary patterns and increase cardiac measurements and vascular calibers, which can predict the degree of cardiorespiratory involvement and establish a prognosis for affected patients. Among the methods used to evaluate the overall dimensions of the cardiac silhouette, vertebral-heart size (VHS) and manubrium-heart size (MHS) are recommended, identifying an increase in more than half of the animals in the sample. Some cases present measurements within the normal range, which may be related to the onset of the patient's clinical worsening or the prepatent period of the infection.

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