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Embolism and lung damage due to *Dirofilaria immitis* in a Dog: Case report

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Abstract
Dirofilaria immitis is a nematode that is expanding in Panama facilitated by the migration of people with their pets, due to tourism or social exodus between countries and also the increase in the ideal conditions for the development of its vectors. The treatment of this disease brings an imminent risk of pulmonary embolism caused by dead parasites in the vascular lumen. Here the canine death due to post-treatment pulmonary embolism is reported. Treatment was carried out with doxycycline, afoxolaner, Milbemycin oxime, prednisolone and melarsomine. Death occurred 19 days after the third dose of melarsomine and when performing the necropsy and histopathological study, the lumen of the small and medium caliber pulmonary blood vessels was observed completely occluded by the nematodes. Multifocal interstitial pneumonitis, pulmonary fibrosis, pulmonary edema, inflammatory lesion of the pulmonary vascular endothelium and focal pericarditis, with a calcified nematode found in the pericardium, were also observed.

Keywords: *Dirofilaria immitis*, embolism, dirofilariosis, heart, lungs, dog

Introduction
Vector diseases are growing exaggeratedly in countries with a warm climate, with climatic changes and global warming largely contributing to the increase in their incidence and prevalence. Vectors, increasingly common pests, are expanding their geographic distribution through adaptive and speciation processes. The etiological agents, increasingly present in the different countries of Latin America, greatly favored by the high human migration due to social factors and the stimulation of tourism, mainly ecological, become increasingly resistant to medications. On the other hand, Panama is a transit country, whether commercial or tourist, which implies the influx of people from other countries, often with their pets. *Dirofilaria immitis* (Spirurida: Onchocercidae), a nematode with wide geographical distribution, also know as heartworm, a zoonotic disease that was described for the first time in Panama in 2021 Chacon and Candanedo [1], is increasing in this country, mainly due to ignorance of its real prevalence, the lack of scientific articles that denounce its pathogenicity and the lack of prevention policies, has a high risk factor in its treatment due to pulmonary embolism caused by the nematodes once they are dead. The objective of this case report is to inform on death due to pulmonary embolism after treatment of *D. immitis* and the damage caused by this parasite to the heart and lungs in a canine.

Case Report.
On October 5, 2022, a canine neutered male, of the Italian Greyhound breed, 9 years old, weighing 5 kg, from the Municipality of Boca Chica, District of San Lorenzo, Province of Chiriqui, Panama was received for consultation at our hospital. The owner brings the pet and reports that the animal has a cough, lack of appetite and fatigue. During the consultation, alterations were observed in cardiorespiratory auscultation showing cardiac arrhythmia, tachypneia, increased capillary perfusion and normal temperature. A blood sample was collected to perform a blood count, which is detailed in the results, and a rapid test called Snap 4dx from Idexx with ELISA technology was performed, resulting positive for *Dirofilaria immitis*. The treatment was prescribed, which the tutor only started 21 days later.

The indicated treatment was Doxycycline at 10 mg per live kilo every 12 hours for 28 days, and a commercial combination of 18.75 mg of Afoxolaner with 3.75 mg of Milbemycin Oxima monthly as a filaricide. On day 30 after starting the treatment, the first deep intramuscular injection of Melarsomine was applied in the lumbar muscles at a dose of 2.5 mg/Kg, associated with oral prednisolone 1 mg/Kg every 12 hours for 7 days, then every 24 hours for 7 more days and every 48 hours for 14 more days until completing 28 days of the steroid, additionally indicating complete rest. On day 60 of starting the treatment, the second injection of Melarsomine was applied, the same as on day 61, and a new prednisolone protocol was started for 28 more days, again emphasizing the necessary rest. On the 80th day after starting the treatment, the animal suddenly died. A necropsy was performed, nematodes were collected, and heart and lungs were fixed in 10% formaldehyde for histopathological evaluation. Macroscopic observation of the pieces and elaborate histological sections stained with Hematoxylin/Eosin for microscopic evaluation were carried out.

The hemogram showed alterations in all blood cells (Table 1). The test revealed relative lymphocytosis and monocytosis. Low hematocrit, low hemoglobin, normochromic microcytic anemia, increased mean erythrocyte size, and thrombocytopenia.

Macroscopic evaluation of the pieces showed the presence of a calcified nematode in the pericardium (Fig 1), without other macroscopic alterations in heart. In the lungs, nematodes were observed throughout the entire course of the right ventricle, right atrium, and pulmonary artery, including invading small and medium caliber vessels in the lung parenchyma. The lungs were hepatized, firm, fibrous when cut, with edematous lesions (Fig 2).

At the histological level, no significant alterations were observed in the heart, only a small focus of pericarditis in resolution, showing normal epicardium, myocardium, endocardium, and heart valves. Completely abnormal histology was observed in lungs, with mild fibrosis of the interstitium and multifocal interstitial nodular lymphocytic/histiocytic infiltrate, with alveoli filled with serous fluid without alterations in pneumocytes, emphysema or presence of particle (Fig 3, 4). Numerous nematodes were observed intravascularly in small and medium caliber vessels at the lung level and endothelial lesion with lymphocytic and histiocytic infiltrate was observed. As well as extensive fibrosis of the tunica media and adventitia at the vascular level. No rupture of the vascular wall or evidence of extravasation of erythrocytes into alveoli or interstitium was observed. No microcalcifications were observed at the alveolar, bronchiolar, bronchial, or vascular level. The vascular lumen was completely occluded by the nematodes (Fig 5, 6, 7) and by the endothelial lesion. The cause of death was pulmonary embolism due to the high parasite load in the lumen of the blood vessels.

Discussions

The damage caused to heart was minimal, only in pericardium where a calcified nematode and a focus of local pericarditis were found as previously reported in the literatura for this disease in Panama Chacon and Candanedo [1,2]. The most aggressive lesions were observed in the vascular wall of the lung parenchyma, lymphocytic-histiocytic infiltrate at the endothelial level as observed by Chacon and Candanedo [2], however without the pseudopapillary hyperplasia observed by this and other authors Chacon and Candanedo [2], Guerrero [3], Ranjbar *et al.* [4], Ceribasi and Simsek [5], Pasca *et al* [6]. The other vascular wall lesions coincide with those reported by Chacon and Candanedo [2], as well as Pneumonitis characterized by multifocal interstitial nodular infiltrate in the parenchyma. However, at the leve of the lower airways, no peribronchiolar infiltrate was observed as cited by the authors [2]. This reinforces our hypothesis that the severity of vascular injury is related to the load and time of parasitism.

Kramer *et al.* [7] reported that the associative treatment of doxycycline and a filaricide before starting treatment with melarsomine showed less severity of pulmonary vascular injury than when compared with doxycycline and melarsomine or melarsomine alone.

Carreton *et al.* [8,9] indicate that some markers can be very reliable indicators for the follow-up and monitoring of pulmonary thromboembolism in canines with *D. immitis* and that these markers that can show inflammation and endothelial damage, as well as pulmonary hypertension, are essential for early detection of damage caused by the disease.

The patient's death occurred 19 days after the third dose of melarsomine, being within the range reported by Carretón and Montoya-Alonso [10], confirming the high risk of post-treatment pulmonary thromboembolism even when taking medication and behavioral measures to minimize this risk.

Competing interests

The authors declare that they have no competing interests.

Author´s contributions

Both authors made substantial contributions to the manuscript. SCCh and PGCC performed the laboratory tests and necropsy. SCCh wrote the manuscript. PGCC made corrections to the text. Both authors read and approved the final manuscript.

Table 1: Whole blood Hemogram collected from a positive dog to *D. immitis*. Mindray BJC2800VET.

WBC (6-17)	10.0 x 10^9/L
Lym# (1-5)	3.9 x 10^9/L
Mon# (0.15-1.35)	1.0 x 10^9/L
Gran# ((4-12.6)	5.1 x 10^9/L
Lym% (12-30) (2-9)	39.3%
Mon%	9.5%
Gran%	51.2%
RBC (5.5-8.5)	4.34 x 10^{12}/L
HGB (12-18)	9.1 g/dL
HCT (37-55)	25.6%
MCV (60-75)	59.2 fL
MCH (20-25)	20.9 pg
MCHC (32-36)	35.5 g/dL
RDW (11-15.5)	16.4%
PLT (200-700)	107 x 10^9/L
MPV	8.0 fL
PDW	17.1
PCT	0.085%
Eos% (1-1.25)	14.4%

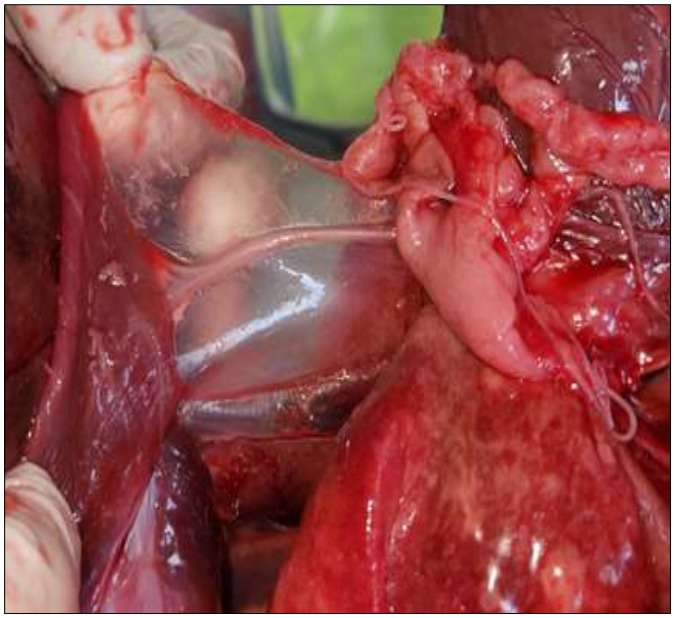

Fig 1: *D. immitis* calcified adult in the pericardium of a canine heart

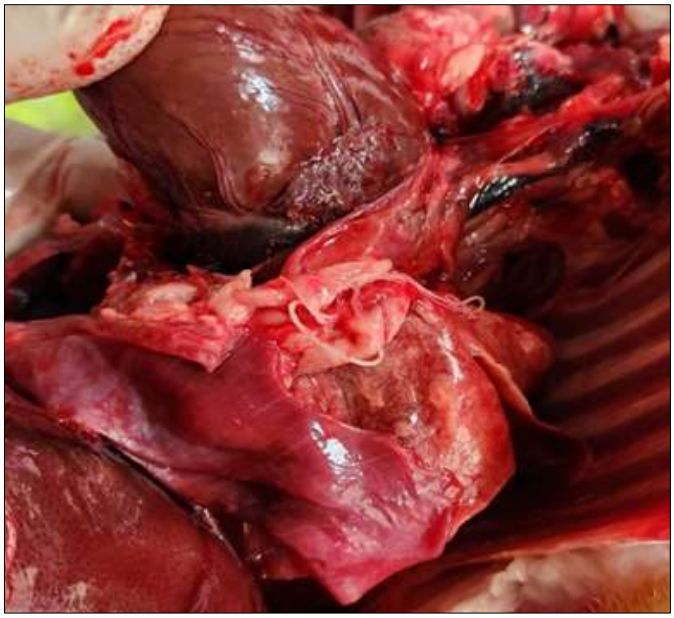

Fig 2: Macroscopic edematous lesion in canine lung parasitized by *D. immitis*. Photo HVHP

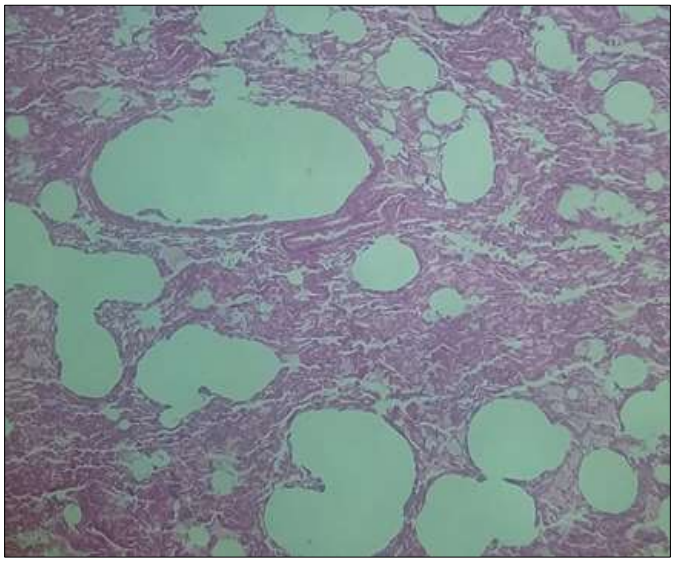

Fig 3: Collapsed canine lung airspaces with edematous interstitium and infiltration of lymphocytes and histiocytes

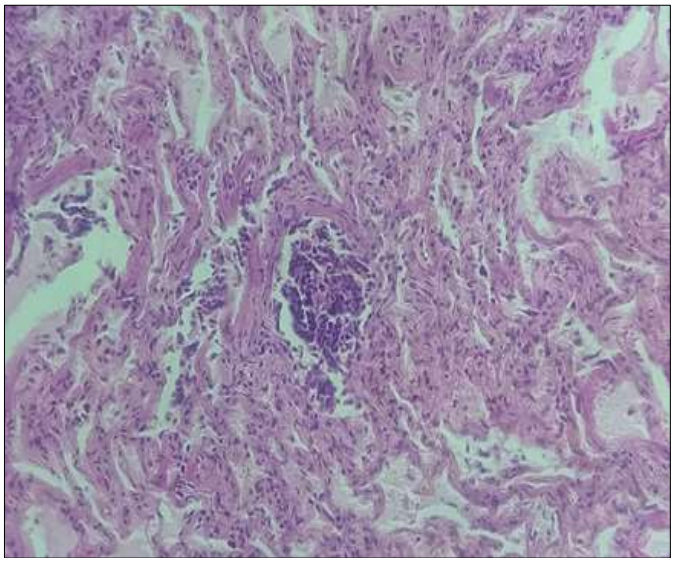

Fig 4: Focus of lymphocytic inflammatory infiltrate in canine lung parenchyma caused by *D. immitis*. Micrograph 200x, Hematoxylin-Eosin. Photo MDofChiriqui

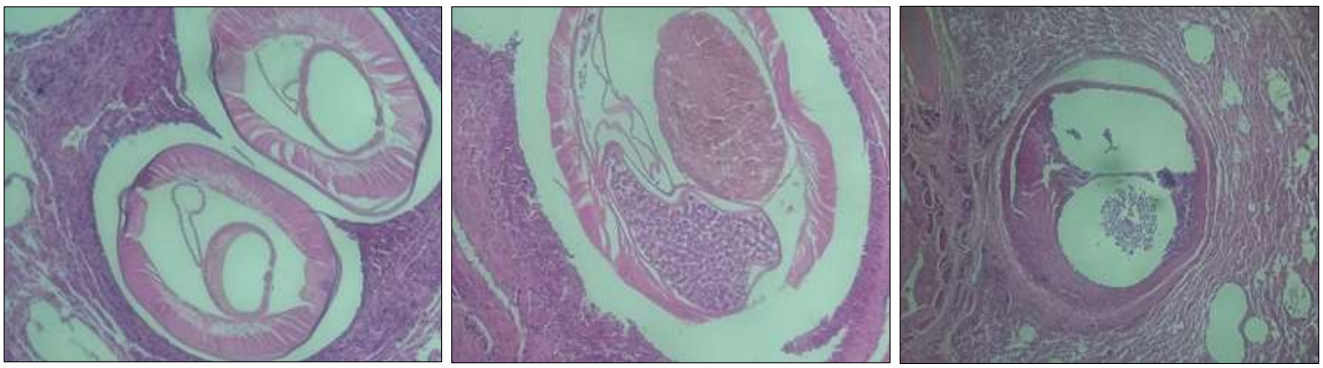

Fig 5, 6, 7: Intravascular nematodes of *D. immitis* in canine lung. Micrograph 200x, Hematoxylin-Eosin. Photo MDofChiriqui

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