

# Unusual pulmonary thromboembolism in a *Dirofilaria immitis* infected dog

Tromboembolismo pulmonar incomum em um cão infectado por *Dirofilaria immitis*

Bruno Alberigi<sup>1\*</sup>, Nathália Marques de Oliveira Lemos<sup>2</sup>, Bernardo Nogueira de Farias<sup>3</sup>, Elan Cardozo Paes de Almeida<sup>4</sup>, Flavya Mendes-de-Almeida<sup>5</sup> & Norma Labarthe<sup>6</sup>

<sup>1</sup>Veterinarian, Dsc. Departamento de Medicina e Cirurgia Veterinária, Universidade Federal Rural do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

<sup>2</sup>Veterinarian. Programa de Pós-graduação em Medicina Veterinária, Universidade Federal Rural do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

<sup>3</sup>Veterinarian. Independent researcher, São Pedro da Aldeia, Rio de Janeiro, Brasil

<sup>4</sup>Veterinarian, Dsc. Departamento de Ciências Básicas, Universidade Federal Fluminense – UFF, Nova Friburgo, RJ, Brasil

<sup>5</sup>Veterinarian, Dsc. Departamento de Patologia e Clínica Veterinária, Universidade Federal Fluminense – UFF, RJ, Brasil

<sup>6</sup>Veterinarian, Dsc. Programa de Pós-graduação Bioética, Ética e Saúde Coletiva, Fundação Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil

## Abstract

The nematode *Dirofilaria immitis* and its endosymbiont *Wolbachia* are the agents of canine heartworm (HW) disease. The worm's main habitats are the pulmonary arteries, which elicit inflammation and disorders of the coagulation process. Clinical signs vary according to multiple factors, including worm burden, individual reaction, and duration of infection. This report describes an unusual case of pulmonary thromboembolism secondary to HW infection. A 7-year-old female dachshund presented with a history of cough, dyspnea, and two syncope episodes. On physical examination, the oral mucosa was slightly discolored, the femoral pulse was weak, respiratory distress was present, and the abdomen distended. Echocardiography showed severe right atrioventricular and pulmonary artery enlargement and a large structure with high echogenicity in the artery. After the dog died naturally, the lungs and heart were examined. Only one worm was found in the right ventricle, and a large thrombus was found in the main pulmonary artery. Histologically, fibrin thrombi and fragments of parasites were present inside the pulmonary artery branches. These findings highlight the importance of a thorough clinical evaluation of HW patients and confirm that only a few worms can threaten an animal's life. Therefore, veterinarians must enforce chemoprophylaxis once treatment is life threatening.

**Keywords:** heartworm, echocardiogram, thrombus, dog.

## Resumo

O nematoide *Dirofilaria immitis* e seu endossimbionte *Wolbachia* são os agentes etiológicos da dirofilariose canina (HW). Os principais habitats do verme são as artérias pulmonares, onde provoca inflamação e distúrbios de coagulação. Os sinais clínicos variam de acordo com vários fatores que incluem carga parasitária, reação individual do hospedeiro e tempo de infecção. O presente relato descreve um caso incomum de tromboembolismo pulmonar secundário à infecção por *D. immitis*. Uma fêmea dachshund de 7 anos apresentou tosse, dispnéia e dois episódios de síncope na última semana. No exame físico, a mucosa oral estava levemente hipocorada, o pulso femoral fraco, presença de dispnéia e distensão abdominal. O ecocardiograma mostrou grave aumento atrioventricular direito e da artéria pulmonar além de uma grande estrutura de ecogenicidade aumentada na artéria pulmonar principal. Após a morte natural do cão, pulmões e coração foram submetidos a uma avaliação macroscópica e microscópica. Apenas um verme foi encontrado no ventrículo direito e um trombo grande foi encontrado na artéria pulmonar principal. Histologicamente, trombos de fibrina e fragmentos de parasitas estavam presentes dentro dos ramos da artéria pulmonar. Esses achados ressaltam a importância de uma avaliação clínica completa dos pacientes com *D. immitis* e confirmam que apenas alguns vermes podem ameaçar a vida de um animal. Portanto, os médicos veterinários devem preconizar a profilaxia, uma vez que a doença e o tratamento da mesma podem ser fatais.

**Palavras-chave:** verme-do-coração, ecocardiograma, trombo, cão.

## Introduction

*Dirofilaria immitis* is a nematode that inhabits the pulmonary vessels and right chambers of the heart that commonly affects dogs through transmission by their vector mosquitoes (Rawlings, 1986).



**How to cite:** Alberigi, B., Lemos, N. M. O., Farias, B. N., Alemida, E. C. P., Mendes-de-Almeida, F. & Labarthe, N. (2020). Unusual pulmonary thromboembolism in a *Dirofilaria immitis* infected dog. *Brazilian Journal of Veterinary Medicine*, 42, e000320. <https://doi.org/10.29374/2527-2179.bjvm000320>

**Financial support:** NMOL – Received scholarship from FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro). BA, BNF, ECPA, FMA, NL – None.

**Conflict of interests:** No conflict of interests declared concerning the publication of this article.

**Received:** October 26, 2020.

**Accepted:** November 03, 2020.

The study was carried out at Laboratório de Quimioterapia Experimental em Parasitologia Veterinária, Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil.

### \*Correspondence

Bruno Alberigi  
Departamento de Medicina e Cirurgia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro – UFRRJ  
Rodovia BR 465, Km 7, Campus Universitário, Bairro Zona Rural  
CEP 23897-000 - Seropédica (RJ), Brasil  
E-mail: bruno.alberigi@gmail.com

Copyright Alberigi et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License which permits unrestricted non-commercial use, distribution, and reproduction in any medium provided the original work is properly cited.

This parasite and its endosymbiont *Wolbachia* are the agents of canine heartworm (HW) disease, which can present asymptomatic to a severe form of the disease depending on the parasitic burden, duration of infection, and individual immunological reactions in the face of the infection (Bowman & Atkins, 2009; Dillon et al., 1995). Disease tends to be more severe in long-term infections with heavy worm burden, and clinical signs may include cough, dyspnea, intolerance to exercise, syncope, ascites, and even death (Rawlings, 1986; McCall et al., 2008; Labarthe et al., 2009; Mircean et al., 2017).

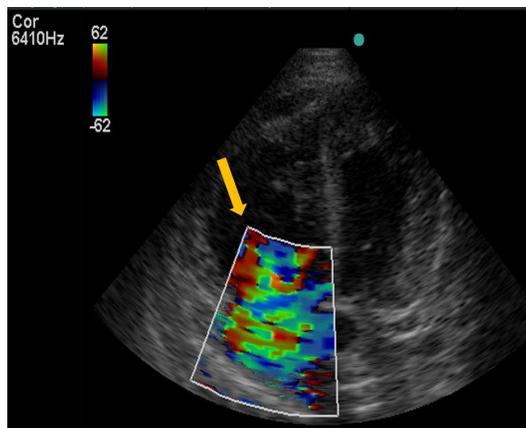
The occurrence of pulmonary thromboembolism (PTE), due to the presence of adult worms, can obliterate the passage of blood from the heart to the lungs and is well known and described, although rare (McHaffie, 2012). The presence of PTE is a life-threatening event due to the possibility of completely obstructing the blood flow to the lungs, although the obstruction can be partial, leading to Cor pulmonale (MacNee, 1994; Haddad et al., 2008; McCall et al., 2008).

Although the presence of the parasite and its endosymbiont, *Wolbachia*, elicits PTE through physical mechanisms and direct inflammation (Rawlings, 1986; Calvert & McCall, 1998), indirect factors may also contribute to its development. These factors include platelet function activation, fibrinolytic activators, and factor Xa, which are common during heartworm infection (González-Miguel et al. 2015; Takashima et al., 2017; Diosdado et al., 2020). The aim of this report was to describe an unusual case of pulmonary thromboembolism secondary to *D. immitis* infection.

## Case description

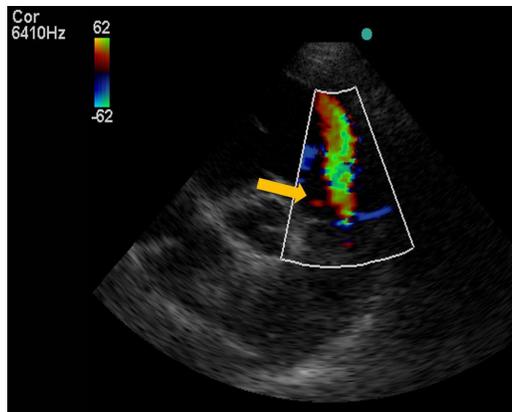
A 7-year-old dachshund spayed bitch weighing 9kg presented with cough and dyspnea for the past seven days. She presented with two syncope episodes during this week. The owner reported that the animal was under alternative treatment for HW disease based on doxycycline and moxidectin. On physical examination, the oral mucosa was slightly discolored, the femoral pulse was weak, respiratory distress was present, and the abdomen distended. Cardiac auscultation revealed the presence of systolic heart murmur grade III/VI at the tricuspid valve area, and diastolic murmur at the pulmonary valve area grade III/VI. Pulmonary auscultation crackle sounds were mainly heard in the right lung. Based on the critical clinical conditions, the patient was submitted to oxygen therapy and additional examinations to determine the main cause of the signs.

Abdominal ultrasound, echodopplercardiography, and electrocardiogram (EKG) were performed immediately. Ultrasound showed hepatomegaly, dilation of the liver's blood vessels, and a mild volume of abdominal fluid. Echocardiography in B-mode showed severe right atrioventricular dilatation, slight hypertrophy of the right ventricular wall and interventricular septum, reduction in the left ventricular lumen, and enlargement of the pulmonary artery. During the examination of the main pulmonary artery, a large structure with high echogenicity was observed. The evaluation with color-flow Doppler demonstrated the presence of severe tricuspid regurgitation (Figure 1) and moderate pulmonary regurgitation (Figure 2). The evaluation using continuous-wave Doppler showed that tricuspid regurgitation had a maximum peak velocity of 4.48 m/s.



→ Shows the presence of severe tricuspid regurgitation.

**Figure 1.** Echocardiographic image in the apical four-chamber parasternal view, showing presence of severe tricuspid regurgitation.



→ shows moderate pulmonary regurgitation.

**Figure 2.** Echocardiographic Color Doppler image of the right ventricular outflow tract showing moderate pulmonary regurgitation.

The EKG (DII lead; N; 50 mm/s) showed the presence of a *p-pulmonale* wave (0.5mV), the presence of an elevated ST segment (0.4 mV), an increase in the T wave amplitude (25% greater than the R wave), the presence of a deep S wave (-2.2 mV), right cardiac axis deviation (between -90° and -120°), and an average heart rate of 150 bpm (Figure 3).



▼ *p-pulmonale* wave;

↑ Elevated ST segment;

→ Increased in the T wave amplitude;

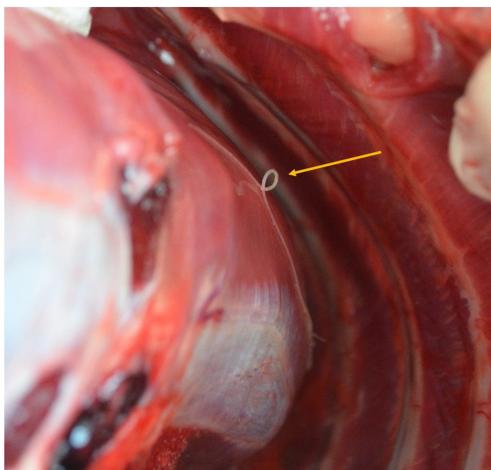
→ Deep S wave.

**Figure 3.** Electrocardiogram (DII lead; N; 50mm/s) showing presence of *p-pulmonale* wave; elevated ST segment, an increase in the T wave amplitude and presence of deep S wave.

Before chest radiography and therapeutic approach could be performed, the patient died due to cardiorespiratory arrest.

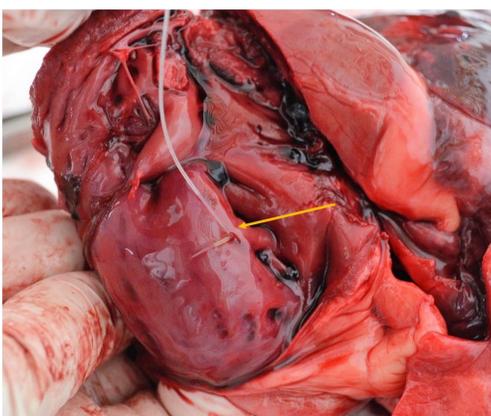
After the owner's consent for *postmortem* examination of the thorax, the thoracic cavity was opened and examined. Only two female worms were found. One worm was found free in the cavity (Figure 4), and the other was found entangled at the *trabeculae carneae* (Figure 5) of the right ventricle. The right chambers of the heart were enlarged, and red-blue areas were observed in the right lobes (superior, middle, and inferior) of the lung (Figure 6). A thrombus of approximately 5cm in length was found in the main pulmonary artery (Figure 7). Lung samples were individually fixed in 10% formaldehyde and sent for histopathological evaluation.

Histologically, the lung parenchyma showed areas of atelectasis contiguous to compensatory emphysema and diffuse thickening of alveolar walls with the presence of macrophages also observed inside the alveolar cavity. Thickening of the artery wall and intima with villous projections was observed in the main pulmonary artery and its branches inside the parenchyma (Figures 8, 9). Necrotic areas surrounded by inflammatory cells and fibroblasts were also observed. Fibrin thrombi, some already organized and recanalized, were present inside pulmonary artery branches in the parenchyma. Fragments of parasites inside the artery branches (Figure 10) and a large fibrin thrombus was seen in the main pulmonary artery (Figure 11).



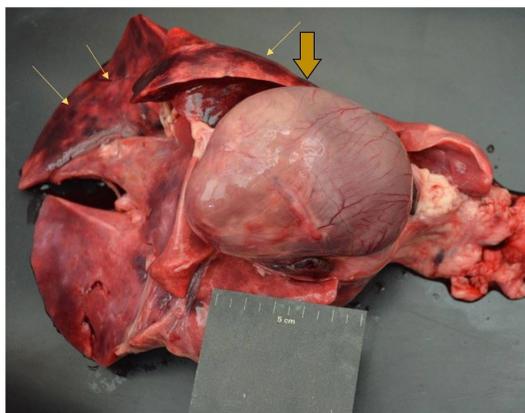
→ Worm free in the cavity.

**Figure 4.** Postmortem examination of the dog which died due to heartworm infection showing a free worm in the thoracic cavity.



→ Entangled worm at the trabeculae carneae.

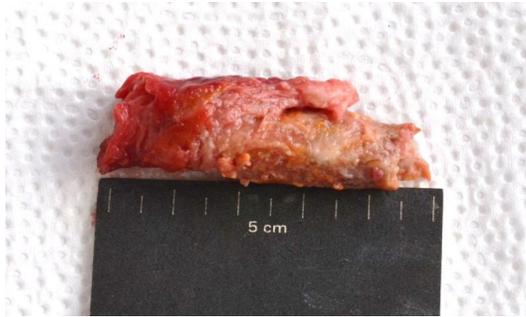
**Figure 5.** Post mortem examination of the dog which died due to heartworm infection showing an entangled worm at the trabeculae carneae of the right ventricle.



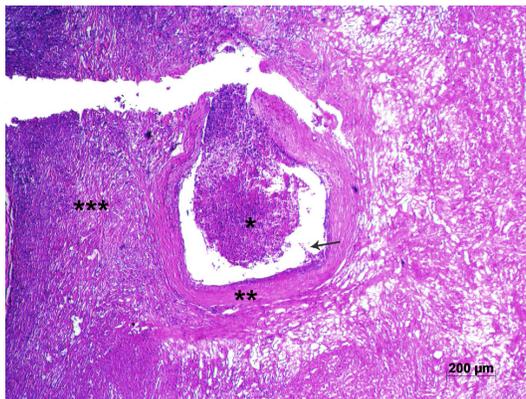
→ Enlargement of cardiac right chambers;

→ Red-blue areas were in the right lobes of the lung.

**Figure 6.** Postmortem examination of the dog which died due to heartworm infection of heart and lungs showing enlargement of cardiac right chambers and red-blue areas in the right lobes of the lung.



**Figure 7.** Thrombus of approximately 5cm long found in the main pulmonary artery of the dog which died due to heartworm infection.



HE Staining;

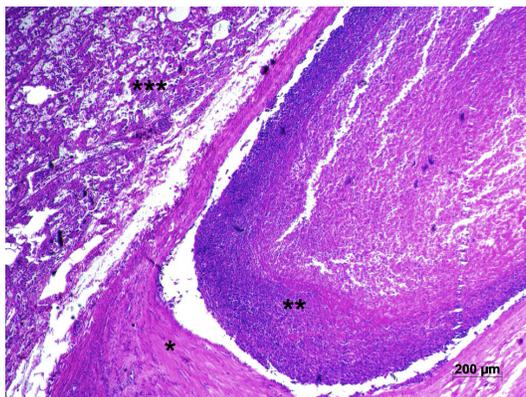
\*Thrombus;

\*\*Thickening of artery wall;

\*\*\*Necrosis area;

→ Villous projection.

**Figure 8.** Lung of the dog which died due to heartworm infection showing thickening of an artery wall, a thrombus attached and villous projection. Extensive necrosis area adjacent to the artery.



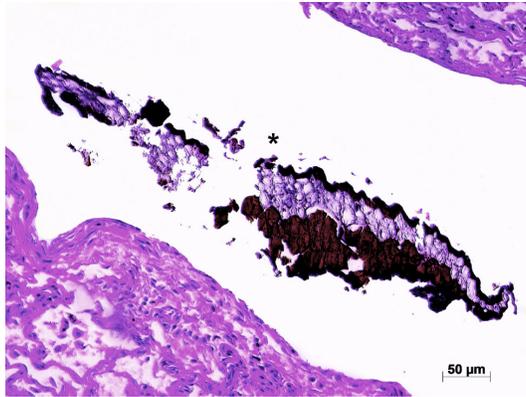
HE Staining;

\*Thickened artery wall;

\*\*Thrombus;

\*\*\*Area of atelectasis.

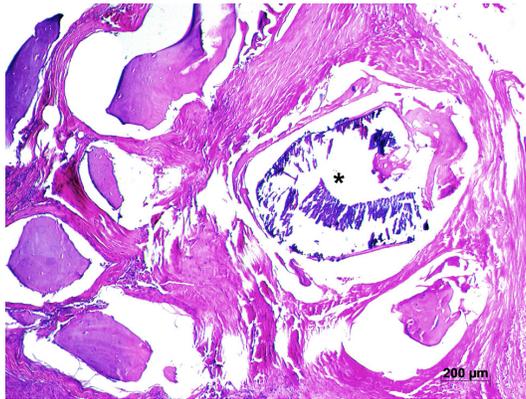
**Figure 9.** Lung of the dog which died due to heartworm infection showing thrombus inside a thickened wall artery. Extensive atelectasis area in the parenchyma adjacent to the artery.



HE Staining;

\*Worm fragment.

**Figure 10.** Lung of the dog which died due to heartworm infection showing a recanalized thrombus showing a worm fragment.



HE Staining;

\*Fragment of parasite.

**Figure 11.** Lung of the dog which died due to heartworm infection showing a fragment of parasite in the lumen of an artery branch.

## Discussion

Although the patient presented with clinical signs compatible with HW infection and with the previous treatment the dog was receiving, further investigation was needed to exclude other causes of heart or lung diseases (American Heartworm Society, 2020) and to evaluate the extension of the lesions.

Physical examination showed signs of decreased cardiac output (discolored oral mucosa, weak femoral pulse, and distended abdomen), suggesting that the pathological process was compromising heart function. However, it was necessary to perform the requested exams to ensure the extent of the lesions and to lead treatment (Gompf, 2008; American Heartworm Society, 2020). Lung sounds detected, despite classically indicating cardiogenic pulmonary edema, when evaluated with the other physical examination findings (abdominal distention, murmur in the pulmonary and tricuspid areas) were not consistent. These findings suggested the inflammatory onset in the lungs, due to HW disease (Atwell, 1988; Ames & Atkins, 2020), since the patient was known to be HW-infected.

Echocardiographic evaluation suggested pulmonary hypertension as a consequence of the thrombus. This was confirmed by the absence of morphological abnormalities at the tricuspid

and pulmonary valve apparatus associated with increased tricuspid regurgitation velocity, enlargement of the main pulmonary artery, and the presented clinical signs (Ames & Atkins, 2020; Reiner et al., 2020). The severity of the case was further reiterated by the presence of a ST-segment elevation on the EKG, an uncommon finding in dogs that reflects the low supply of oxygen to the cardiac muscle and may indicate rare canine myocardial ischemia (Santilli et al., 2020).

It is well known that HW disease therapy can present systemic complications. When worms die, there is intense inflammatory stimulus due to the worm's and *Wolbachia*'s antigens that trigger pulmonary reactions and can even elicit verminotic embolism (Rawlings, 1986; Turner et al., 2009; Kramer & Genchi, 2014). However, this correlation cannot be either affirmed or excluded in the present case since the animal's history is veiled. Therefore, to increase the capacity to choose the best adulticidal treatment regimen to reduce the risk for the patient, a complete assessment of the patient's health condition must be performed. A thorough clinical evaluation must always be performed, including blood count, biochemistry, and radiography. Echocardiography may be also needed (American Heartworm Society, 2020). Although worm burden is directly related to the clinical manifestations (Dillon et al., 1995; Bowman & Atkins, 2009), in the present case only one adult worm was found inside the heart, in addition to the low number of parasite fragments found inside the large fibrin thrombus, suggesting that the severity of the disease was due to the patient's inflammatory reaction to injury (Ames & Atkins, 2020), more than to the parasite burden. In association with the main lesions, thickening of the alveolar wall must have reduced alveolar complacency, resulting in emphysema (Sutton & Atwell, 1985), reducing the air passage leading to atelectasis (West, 2014).

The presence of worms and their endosymbionts can cause coagulation disorders that are associated with endothelial injury, favoring thrombus formation (González-Miguel et al., 2015; Takashima et al., 2017; Diosdado et al., 2020). As there are no hematological work results available, it is impossible to include or exclude coagulation disorder as the primary cause of thrombi formation. Nevertheless, the number of thrombi found in the pulmonary arteries, the rapid onset of disease, and clinical worsening of the patient's health strongly suggest that coagulation disorders play an important role.

The presence of an inflammatory process is expected because the presence of the worms and its endosymbiont trigger the host's immune system and predispose thromboembolic events (Rawlings, 1986; Carretón et al., 2013; Yoon et al., 2017). Small fragments of the thrombus may have got detached and reached the pulmonary artery branches as identified by microscopy. This process presumptuously was the main cause of tissue ischemia, translated in the form of necrotic areas (Shapiro, 2009) as identified. The presence of recanalization reflects the attempt of reperfusion of the lung in the face of ischemia, suggesting that the obstruction of these vessels preceded the total obstruction of the main pulmonary artery (Wagenvoort, 1995).

The presence of parasite fragments inside the thrombus suggests the presence of at least a third parasite. It is possible to infer that, due to its location, its presence was a determinant of thrombus formation. The presence of the endothelial lesion observed, associated with platelet activation and reduction of thrombolytic mechanisms expected in HW infections may have contributed to thrombogenesis (González-Miguel et al., 2015; Takashima et al., 2017; Falcón-Cordón, et al., 2019; Diosdado et al., 2020).

## Conclusions

These findings highlight the importance of a thorough clinical evaluation of HW-infected patients before any adulticidal treatment. This case confirms that only few worms are enough to threaten an animal's life. Therefore, small animal practitioners must always enforce chemoprophylaxis to avoid HW infection; once treatment, even in the face of a small worm burden, is life threatening.

## Acknowledgements

The authors thank Maria Isabel Rossi for the consulting in the field of pathology and Celeste da Silva Freitas de Souza and Natalia Freitas de Souza for performing the photomicrographies.

## References

- American Heartworm Society - AHS. (2020). *Current canine guidelines for the prevention, diagnosis and management of heartworm (Dirofilaria immitis) infection in dogs*. USA: AHS. Retrieved in 2020, August 21, from [https://d3ft8sckhnqim2.cloudfront.net/images/pdf/2020\\_AHS\\_Canine\\_Guidelines.pdf?1580934824](https://d3ft8sckhnqim2.cloudfront.net/images/pdf/2020_AHS_Canine_Guidelines.pdf?1580934824)
- Ames, M. K., & Atkins, C. E. (2020). Treatment of dogs with severe heartworm disease. *Veterinary Parasitology*, 283, 109131. <http://dx.doi.org/10.1016/j.vetpar.2020.109131>. PMID:32521392.
- Atwell, R. B. (1988). Clinical signs and diagnosis of canine dirofilariasis. In: P. F. L. Boreham & R. B. Atwell (Eds.), *Dirofilariasis* (pp. 61-81). Boca Raton: CRC Press.
- Bowman, D. D., & Atkins, C. E. (2009). Heartworm biology, treatment, and control. *Veterinary Clinical small Animal Practice*, 39(6), 1127-1158. <http://dx.doi.org/10.1016/j.cvsm.2009.06.003>.
- Calvert, C. A., & McCall, J. W. (1998). Canine heartworm disease In P. R. Fox (Ed.), *Canine and feline cardiology* (pp. 519-549). USA: Saunders.
- Carretón, E., Morchón, R., González-Miguel, J., Simon, F., Juste, M. C., & Montoya-Alonso, J. A. (2013). Variation of D-dimer values as assessment of pulmonary thromboembolism during adulticide treatment of heartworm disease in dogs. *Veterinary Parasitology*, 195(1-2), 106-111. <http://dx.doi.org/10.1016/j.vetpar.2013.01.005>. PMID:23384581.
- Dillon, A. R., Brawner, W. R., & Hanrahan, L. (1995). Influence of number of parasites and exercise on the severity of heartworm disease in dogs. In *Proceedings of the American Heartworm Society Symposium*. Batavia: American Heartworm Society.
- Diosdado, A., Simón, F., Morchón, R., & González-Miguel, J. (2020). *Dirofilaria immitis* possesses molecules with anticoagulant properties in its excretory/secretory antigens. *Parasitology*, 147(5), 559-565. <http://dx.doi.org/10.1017/S0031182020000104>. PMID:31992384.
- Falcón-Cordón, Y., Montoya-Alonso, J. A., Caro-Vadillo, A., Matos-Rivero, J. I., & Carretón, E. (2019). Persistence of pulmonary endarteritis in canine heartworm infection 10 months after the eradication of adult parasites of *Dirofilaria immitis*. *Veterinary Parasitology*, 273, 1-4. <http://doi.org/10.1016/j.vetpar.2019.07.008>.
- Gompf, R. The history and physical examination (2008). In F. W. K. Smith Junior, L. P. Tilley, M. A. Oyama, & M. M. Sleeper (Eds.), *Manual of canine and feline cardiology* (4th ed., pp. 2-23). USA: Saunders. <http://dx.doi.org/10.1016/B978-141602398-2.10001-4>.
- González-Miguel, J., Morchón, R., Siles-Lucas, M., & Simón, F. (2015). Fibrinolysis and proliferative endarteritis: two related processes in chronic infections? The model of the blood-borne pathogen *Dirofilaria immitis*. *PLoS One*, 10(4), e0124445. <http://dx.doi.org/10.1371/journal.pone.0124445>. PMID:25875022.
- Haddad, F., Doyle, R., Murphy, D. J., & Hunt, S. A. (2008). Right ventricular function in cardiovascular disease, part II: pathophysiology, clinical importance, and management of right ventricular failure. *Circulation*, 117(13), 1717-1731. <http://dx.doi.org/10.1161/CIRCULATIONAHA.107.653584>. PMID:18378625.
- Kramer, L., & Genchi, C. (2014). Where are we with *Wolbachia* and doxycycline: an in-depth review of the current state of our knowledge. *Veterinary Parasitology*, 206(1-2), 1-4. <http://dx.doi.org/10.1016/j.vetpar.2014.03.028>. PMID:24813786.
- Labarthe, N., Paiva, J., & Campos, J. (2009). *Dirofilariose*. In C. B. Marcondes (Eds.), *Doenças transmitidas e causadas por artrópodes* (pp. 283-299). Ribeirão Preto: Atheneu.
- MacNee, W. (1994). Pathophysiology of cor pulmonale in chronic obstructive pulmonary disease. Part two. *American Journal of Respiratory and Critical Care Medicine*, 150(4), 1158-1168. <http://dx.doi.org/10.1164/ajrccm.150.4.7921453>. PMID:7921453.
- McCall, J. W., Genchi, C., Kramer, L. H., Guerrero, J., & Venco, L. (2008). Heartworm disease in animals and humans. *Advances in Parasitology*, 66(1), 193-285. [http://dx.doi.org/10.1016/S0065-308X\(08\)00204-2](http://dx.doi.org/10.1016/S0065-308X(08)00204-2). PMID:18486691.
- McHaffie, J. (2012). *Dirofilaria immitis* and *Wolbachia pipientis*: a thorough investigation of the symbiosis responsible for canine heartworm disease. *Parasitology Research*, 110(2), 499-502. <http://dx.doi.org/10.1007/s00436-011-2644-5>. PMID:21922235.
- Mircean, M., Ionica, A. M., Mircean, V., Gyorke, A., Codea, A. R., Tabaran, F. A., Taulescu, M., & Dumitrache, M. O. (2017). Clinical and pathological effects of *Dirofilaria repens* and *Dirofilaria immitis* in a dog with a natural co-infection. *Parasitology International*, 66(3), 331-334. <http://dx.doi.org/10.1016/j.parint.2017.02.003>. PMID:28232044.
- Rawlings, C. A. (1986). *Heartworm disease in dogs and cats*. USA: Saunders.
- Reinero, C., Visser, L. C., Kelliham, H. B., Masseau, I., Rozanski, E., Clercx, C., Williams, K., Abbott, J., Borgarelli, M., & Scansen, B. A. (2020). ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. *Journal of Veterinary Internal Medicine*, 34(2), 549-573. <http://dx.doi.org/10.1111/jvim.15725>. PMID:32065428.
- Santilli, R., Moïse, N. S., Pariaut, R., & Perego, M. (2020). Formação e interpretação das ondaseletrocardiográficas. In R. Santilli, N. S. Moïse, R. Pariaut & M. Perego (Eds.), *Eletrocardiografia de cães e gatos diagnóstico de arritmias* (2. ed., pp. 35-70). São Paulo: MedVet.
- Shapiro, L. Cell Injury and cell death (2009). In L. Shapiro & S. Leland. *Pathology & parasitology for veterinary technicians* (2nd ed., pp. 8-21). Boston: Cengage Learning.

- Sutton, R. H., & Atwell, R. B. (1985). Lesions of pulmonary pleura associated with canine heartworm disease. *Veterinary Pathology*, 22(6), 637-639. <http://dx.doi.org/10.1177/030098588502200620>. PMID:4082390.
- Takashima, Y., Onoda, I., Chiou, S.-P., & Kitoh, K. (2017). *In vitro* canine platelet aggregation caused by *Dirofilaria immitis* extract. *The Journal of Veterinary Medical Science*, 79(2), 387-392. <http://dx.doi.org/10.1292/jvms.16-0461>. PMID:28049921.
- Turner, J. D., Langley, R. S., Johnston, K. L., Gentil, K., Ford, L., Wu, B., Graham, M., Sharpley, F., Slatko, B., Pearlman, E., & Taylor, M. J. (2009). *Wolbachia* lipoprotein stimulates innate and adaptive immunity through Toll-like receptors 2 and 6 to induce disease manifestations of filariasis. *The Journal of Biological Chemistry*, 284(33), 22364-22378. <http://dx.doi.org/10.1074/jbc.M901528200>. PMID:19458089.
- Wagenvoort, C. A. (1995). Pathology of pulmonary thromboembolism. *Chest*, 107(1, Suppl), 10S-17S. <http://dx.doi.org/10.1378/chest.107.1.Supplement.10S>. PMID:7813322.
- West, J. (2014). Doenças vasculares. In J. B. West (Eds.), *Fisiopatologia pulmonar: princípios básicos* (8. ed., pp. 122-147). Porto Alegre: Artmed.
- Yoon, W. K., Kim, Y. W., Suh, S. L., Choi, R., Lee, S. G., & Hyun, C. (2017). Evaluation of cardiopulmonary and inflammatory markers in dogs with heartworm infection during treatment with the 2014 American Heartworm Society recommended treatment protocol. *Parasites & Vectors*, 10(Suppl 2), 535. <http://dx.doi.org/10.1186/s13071-017-2427-7>. PMID:29143684.