Report of *Dirofilaria immitis* infection with acute cardiopulmonary complications in a cat from Northeastern Brazil

*Relato de infecção por Dirofilaria immitis com complicações cardiopulmonares agudas em um gato no Nordeste do Brasil*

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**ABSTRACT**

Dirofilariasis is a zoonotic disease caused by *Dirofilaria immitis*, a nematode found mainly in the pulmonary artery and right chambers of the heart, lungs, and large vessels of dogs. This parasitism also occasionally occurs in cats, causing an amicrofilaremic and asymptomatic infection, resulting in severe illness and rapid death. In this case report, it was described acute clinical signs and histopathological alterations in a domestic cat with heartworm disease from the city of Mossoró, the Rio Grande do Norte, Brazil. The nematode species, *D. immitis*, was confirmed by morphological and molecular analyses. This is the first documented and full report of feline heartworm disease in northeastern Brazil.

**Keywords:** Parasitic diseases. Onchocercidae. Heartworm. Dirofilariasis.

**RESUMO**

A dirofilariose é uma doença zoonótica causada por *Dirofilaria immitis*, um nematódeo que parasita cães, principalmente a artéria pulmonar e as câmaras direitas do coração, pulmões e grandes vasos. Este parasita também ocorre ocasionalmente em gatos, geralmente causando uma infecção amicrofilarêmica e assintomática, que pode resultar em doença grave e morte rápida. Neste relato de caso, são apresentados os sinais clínicos agudos e alterações histopatológicas em um gato doméstico do município de Mossoró, Rio Grande do Norte, Brasil, com dirofilariose. A espécie de nematóide, *D. immitis*, foi confirmada por análises morfológicas e moleculares. Este é o primeiro relato documentado e completo de dirofilariose felina no Nordeste do Brasil.

**Palavras-chave:** Doenças parasitárias. Onchocercidae. Verme cardíaco. Dirofilariosis.
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Introduction

Dog heartworm (Dirofilaria immitis) is an important Onchocercidae nematode with worldwide distribution. This parasite affects the heart and large vessels of dogs, wild carnivores, and pinnipeds (Alho et al., 2017; Otranto & Deplazes, 2019). Other animals, such as domestic cats and ferrets, are also susceptible to D. immitis infection (Ames, 2018). However, most of the larvae of this nematode do not reach their adult form in these hosts, as it is better adapted to dogs (Lee & Atkins, 2010).

In cats, the death of D. immitis larvae results in an intense inflammatory response related to Wolbachia spp. endosymbiosis, which can be responsible for early signs of heartworm infection, also known as heartworm-associated respiratory disease. This stage of the disease may be mistakenly confused with feline asthma. Domestic cats have potentially life-threatening complications when infected by D. immitis, but unlike what happens in dogs, right-sided heart failure and vena cava syndrome have been rarely documented in these animals (McCall et al., 2008). Thus, this case report aimed to describe a cat from the city of Mossoró, Rio Grande do Norte State, in the Brazilian Caatinga Biome, with acute cardiopulmonary complications due to natural infection by D. immitis.

CASE REPORT

A one-year-old, 3.5-kg, neutered, male mixed-breed cat was admitted for emergency clinical care at the Veterinary Hospital of the Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró city, Rio Grande do Norte State, Brazil. The animal was native to the region and had spent most of his time outside the house, and therefore in contact with other cats, and was not vaccinated, not dewormed, and fed ad libitum with commercial cat food and natural food. Two days before the hospital admission, he presented acute dyspnea, anorexia, and oligodipsia with acute onset. Neutering had been performed seven days before the onset of symptoms. On physical examination, the animal was placed in a sternal position with apatic behavior. The cat was hypothermic, and the heart auscultation revealed hypoponosis of the heart and pulmonary sounds and the probable presence of pleural effusion. The animal showed restrictive expiratory dyspnea, concomitant with superficial tachypnea. Other semiological changes were not observed. The patient was very excited at the time of the physical examination, avoiding excessive manipulation. It is not possible to carry out complementary exams, such as blood count, serum biochemistry, or radiographs of the thoracic cavity.

Thoracocentesis was performed, and yellowish pleural effusion was extracted. Physicochemical analysis of the fluid demonstrated the presence of modified transudate. On the same day, the animal died and necropsy was performed.

A large amount of yellowish turbid liquid was observed in the thoracic cavity (Figure 1A). The lungs were reddish, compact, and foamy fluid was present in the trachea and bronchi. The macroscopic examination of the heart revealed thin and flaccid, with right ventricular and atrial enlargement. A single specimen of an elongated whitish nematode was found in the right ventricle and atrium with morphology suggestive of D. immitis (Figure 1B). This nematode was collected, stored in absolute ethanol, and sent for taxonomic identification and molecular characterization.

The nematode was a mature adult female, measuring 11 cm in length, with a thin and rounded cephalic extremity and posterior part slightly curved. Its cuticle presented a delicate transversal striation along the entire nematode body. The oral opening was rounded, without visible lips. The nerve ring was located at the beginning of the esophagus, at 260 μm from the anterior ending. Excretory pore was not observed. The esophagus was divided into muscular and glandular portions, but without a clear distinction between them, measuring 610 μm in length. The width of the body at the esophageal-intestine junction was 460 μm. The vulvar opening was elongated, without any cuticular ornamentation, located at 1.61 mm from the anterior ending. The anus was simple and subterminal, at 170 μm from the tail tip. The morphological analysis confirmed the nematode species as D. immitis (Figures 1C and D).
Histopathological results showed interstitial edema and peribronchial mononuclear cell infiltrates in the lungs (Figure 1E). Areas of the right heart chambers showed muscle fibers slightly disorganized, but the endocardium remained unchanged. The liver presented mild to moderate lipid vacuolation in the cytoplasm of hepatocytes (Figure 1F). The other organs did not present histopathological alterations. The histopathological diagnosis was chronic broncho-interstitial pneumonia, bronco-interstitial and alveolar pulmonary edema, and hepatic lipidosis (Figure 1).

Nematode DNA was extracted using the protocol proposed by Bag et al. (2016). A DNA sample was submitted to amplification of the 18S rDNA region by using the primers 1813F/2646R (Holterman et al., 2006). MyoHC and hsp70 housekeeping genes were amplified using the primers designed by Lefoulon et al. (2015). The amplicons obtained were sequenced in the ABI3130 sequencer (Applied Biosystems). Generation of consensus sequences of each genomic region amplified and sequence trimming was performed by using the Phred/Phrap/Consed (Gordon et al., 1998). The sequences of 18S rDNA (505 bp), myoHC (390 bp), and hsp70 (371 bp) of the nematode and sequences from the GenBank were aligned separately, using the tool MUSCLE (Edgar, 2004). The aligned sequences of the three genomic
regions were concatenated, and a phylogenetic Bayesian analysis was performed using the MrBayes 3.2.3 software (Ronquist & Huelsenbeck, 2003). The analysis was performed using the best fit model TrN+I+G, according to the Akaike Information Criterion (AIC) (Posada & Buckley, 2004), in four chains with 1,000,000 generations.

The phylogenetic analysis of the 18S rDNA, myoHC, and hsp70 genes concatenated datasets were compatible with *D. immitis* (Figure 2). The accession numbers of the sequences used in this study are presented in Table 1.

**Discussion**

Feline heartworm disease has been reported in many parts of the world, but its particularities are less known in cats than in dogs. This is because definitive *ante-mortem* diagnosis is difficult to achieve, and most feline dirofilariasis infections are asymptomatic. Therefore, the real prevalence of this disease in cats is underestimated in endemic areas (Venco et al., 2008). Unlike in dogs, microfilariae are produced only in 20% of cats with mature female and male worms, whereas they usually remain only a few months in

![Figure 2 – Bayesian phylogenetic tree of concatenated 18S rDNA, myoHC, and hsp70 sequences of the evaluated *Dirofilaria immitis* specimen (in bold) in comparison with other Onchocercidae nematodes. *Protospirura muricola* and *Filaria alata* were used as outgroups.](image_url)

**Table 1 – Accession numbers of Onchocercidae sequences of 18S rDNA, myoHC, and hsp70 genes were used in the phylogenetic analysis**

| Onchocercidae species                  | Code    | 18S rDNA    | myoHC     | hsp70     |
|---------------------------------------|---------|-------------|-----------|-----------|
| Acanthocheilonema odendhali           | 401YU   | KP760116    | KP760212  | KP760410  |
| Cercopithifilaria bainae              | 9YT     | KP760123    | KP760219  | KP760417  |
| Cercopithifilaria rugosicauda         | 350YU   | KP760124    | KP760220  | KP760418  |
| Dipetalonema caudispina               | 64YT    | KP760127    | KP760223  | KP760421  |
| Dipetalonema gracile                  | 63YT    | KP760130    | KP760226  | KP760424  |
| Dirofilaria immitis                   | 79YT    | KP760133    | KP760229  | KP760427  |
| Dirofilaria immitis                   | Dim-cat-RN | MW010021 | MW036532  | MW036533  |
| Dirofilaria repens                    | 297YU   | KP760134    | KP760230  | KP760428  |
| Filaria latale                        | 62YT    | KP760135    | KP760231  | KP760429  |
| Litomosoides brasiiliensis            | 37PF    | KP760140    | KP760236  | KP760434  |
| Loxodontofilaria caprine              | YG2-58  | KP760144    | KP760240  | KP760438  |
| Mansonella perforata                  | 216JW   | KP760145    | KP760241  | KP760439  |
| Mansonella ozzardi                    | 77YT    | KP760147    | KP760243  | KP760441  |
| Onchocerca armillata                  | 54FKa1  | KP760153    | KP760248  | KP760446  |
| Onchocerca dewittei japonica          | OB9 242zpl | KP760154 | KP760249  | KP760447  |
| Oswaldofilaria chabaudi               | 191YU   | KP760159    | KP760254  | KP760452  |
| Protospirura muricola                 | 97YT    | KP760162    | KP760257  | KP760455  |
| Setaria tundra                        | 71YT    | KP760165    | KP760260  | KP760458  |
circulation and at a low number of specimens (Ciuca et al., 2020).

In Europe, the prevalence of feline dirofilariasis varies between different regions. In Barcelona, Spain, it corresponds to about 10% of the prevalence in dogs within the same area (Venco et al., 2011). In the metropolitan area of Zaragoza, also in Spain, the seroprevalence of *D. immitis* in the feline population corresponds to 25.2% (Villanueva-Saz et al., 2021). In Romania, dirofilariasis prevalence varied from 13.4% to 23.0% (Anghel et al., 2016; Girdan et al., 2015; Ionita et al., 2012; Pana et al., 2018). In Tuscany, Italy, it was about 24.9% (Magi et al. 2002) and in the central and northern regions of Portugal, 15.0% of the evaluated cats presented anti-*D. immitis* and anti-*Wolbachia* antibodies (Vieira et al., 2015). A higher prevalence of dirofilariasis in cats has been reported in Central and South America, with 42% being found in the cities of the Gulf Coast of Mexico, and 63.2% in the Caribbean region (Bahamas, Curacao, Cuba, the Dominican Republic, and Puerto Rico). In Florida, necropsies performed on 630 adult cats revealed the presence of heartworms in 4.9% of the animals, with serological evidence of heartworm pre-exposure in 17% of the tested population (Levy et al., 2003).

In South America, some studies have reported lower levels of exposure in cats living in areas where canine dirofilariasis is endemic. Therefore, the risk of *D. immitis* infestation in cats within the same geographic area may partly vary (Dantas-Torres & Otranto, 2013). Indeed, information about the geographical distribution and epidemiological features of *D. immitis* infection in cats is scarce, particularly in the urban stray cat population living in endemic regions for canine heartworm disease (Villanueva-Saz et al., 2021). Specifically in Brazil, there are only two reports of feline heartworm disease (Alberigi et al., 2020; Branco et al., 2009), and both occurred in Rio de Janeiro State, southeastern Brazil.

Histological alterations in the lungs of the studied cat were marked by the presence of eosinophil infiltrates in the parenchyma, pulmonary vasculature, and air spaces, due to pneumonitis caused by the parasite. The pronounced bronchial reactivity was due to the activity of pulmonary intravascular macrophages, a component of the reticuloendothelial system in cats (Dillon et al., 2008).

Acute death of asymptomatic heartworm-infected cats has been widely reported (Holmes, 1993; Dillon, 1984; Ralston et al., 1998), and it has been attributed to acute pulmonary thromboembolism due to spontaneous death of the adult heartworms (Dvorak et al., 2000). The present case showed only one adult heartworm in the right ventricle and atrium, without any obstruction of large vessels, even though clinical signs of dyspnea were observed due to pleural effusion that probably resulted from the inflammatory response of the lungs (Litster & Atwell, 2006). The pulmonary inflammatory process leads to changes in vascular permeability, resulting in the extravasation of proteinaceous fluid from the intravascular environment into the thoracic cavity, causing fluid accumulation (Ames, 2018), which could have justified the origin of the observed pleural effusion. Severe dyspnea, along with reduced blood oxygen saturation and decreased blood pressure are the main signs of *D. immitis* infection in cats (Litster & Atwell, 2006). The dirofilariasis in cats is primarily a lung disease (even adopting a new designation: dirofilariasis-associated respiratory disease), so the main morphological changes are those related to the lungs (Nelson, 2012). Such citation can justify, for the present case, the predominance of histopathological alterations directed to the lungs. The parasite causes an intense inflammatory reaction and thromboembolism, which can lead to acute lung injury. There is subsequent hyperplasia of alveolar cells, which sometimes causes permanent lung dysfunction. Inflammation is enough to constrict the small airways in the lungs (Nelson, 2012).

In the GenBank, there is a lack of *D. immitis* sequences, as well as observed for other Onchocercidae nematodes. The sequencing of the 18S rDNA region alone is not enough for the specific identification of Onchocercidae nematodes (Lefoulon et al., 2015). Thus, the use of molecular biology was important to detect the real existence of *D. immitis* in the studied cat and alert to the possibility of heartworm disease in the region in question, despite the absence of epidemiological surveys for such parasitic disease, in the feline population of the geography region reported. In the city of Mossoro (Rio Grande do Norte State, Brazil) there are only studies aimed at detecting *D. immitis* in dogs, revealing an incidence of 15% to 21%, with such values coming from other diagnostic exams, such as Knott’s test, immunoassay, and necropsy (Batista et al., 2008, 2021), with a lack of knowledge about the use of molecular methods to date. It is known that the prevalence of dirofilariasis in animals occurs more commonly in tropical or subtropical coastal regions, which have favorable climate, temperature, and humidity conditions for the perpetuation of potential vectors for the transmission of *D. immitis*, which corresponds to mosquitoes of the Culicidae family (Leite et al., 2006). The municipality of the report in question has a semi-arid climate and is approximately 40 km away from the coastal areas. Therefore, the record of the case became somewhat uncommon, because the geographic location and the animal species affected are not usually related to
the onset of dirofilariaisis. For the case in question, since the animal is native to a city without a coastal strip, one of the transmission hypotheses could correspond to the presence of other mechanical vectors for *D. immitis*, which have not yet been identified.

### Conclusion

In this case report, the signs observed at necropsy of this feline patient were probably caused by *D. immitis*, since the parasite was identified by morphological and molecular analyses of three genomic regions. Besides describing clinical signs of dirofilariaisis in a cat this case report provided sequences of *D. immitis* that may contribute to molecular diagnostic of the parasite.

### Conflict of Interests

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

### Ethics Statement

The owner of the feline patient provided written informed consent for both diagnostic assessment and publication of this case report, with accompanying images.

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