



Clinical Reports

## First canine dirofilariosis report (*Dirofilaria immitis*) in Campo Grande, Mato Grosso do Sul, Brazil

Primeiro relato de dirofilariose canina (*Dirofilaria immitis*) em Campo Grande, Mato Grosso do Sul, Brasil.

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### ABSTRACT

Dirofilariosis is an emerging zoonosis with a wide distribution worldwide. In Brazil, it is caused by the nematode, *Dirofilaria immitis*, and transmitted by different species of culicid mosquitoes. The present work aimed to report the first case of canine dirofilariosis in the municipality of Campo Grande, Mato Grosso do Sul. On September 6, 2018, a 14-year-old female canine poodle with a history of traveling to the coastal region was seen at a private veterinary clinic in Campo Grande, with hypertension and eosinophilia. The animal was medicated with dipyrone and anlodipine for control of the hypertension and, after nine days, it returned with worsening of the clinical picture (ênese, melena, taquicardia, taquipneia). Complementary laboratory tests were requested, observing cardiac, hepatic, renal and pancreatic alterations, besides the presence of microfilaria, morphologically identified and confirmed as *D. immitis* by serological and molecular tests. The animal was hospitalized, but died on the fifth day after hospitalization. The fact that the animal has a report of a trip to the coastal region, without prophylactic treatment, characterizes the case as an allochthonous of dirofilariosis in the region.

### RESUMO

A dirofilariose é uma zoonose emergente de ampla distribuição mundial. No Brasil, é causada pelo nematódeo, *Dirofilaria immitis*, e transmitida por diferentes espécies de mosquitos culicídeos. O presente trabalho teve por objetivo relatar o primeiro caso de dirofilariose canina no município de Campo Grande, Mato Grosso do Sul. No dia 6 de setembro de 2018, um canino, fêmea, poodle, de 14 anos de idade, com histórico de viagem para região litorânea, foi atendido em uma clínica veterinária particular de Campo Grande, apresentando hipertensão arterial e eosinofilia. O animal foi medicado com dipirona e anlodipino para controle da hipertensão arterial e, após nove dias, retornou com piora do quadro clínico (ênese, melena, taquicardia, taquipneia). Exames laboratoriais complementares foram solicitados, observando-se alterações cardíacas, hepáticas, renais e pancreáticas, além da presença de microfilárias, identificadas morfologicamente e confirmada como *D. immitis* por teste sorológico e molecular. O animal foi internado, mas veio a óbito no quinto dia após internação. O fato de o animal possuir relato de viagem para região litorânea, sem realização de tratamento profilático, caracteriza o caso como alóctone de dirofilariose na região.

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## INTRODUCTION

Dirofilariosis, also popularly known as "heartworm disease", is a neglected emerging zoonosis whose etiological agent is *Dirofilaria immitis* (*D. immitis*), and whose transmission is vectorial. It is a chronic disease with cardiopulmonary involvement in dogs (SEVIMLI et al., 2007).

In the dogs, the disease is of difficult clinical diagnosis, because the clinical signs depend on the number of adult parasites present, the time of evolution of the disease, and the immune response of the host. Clinically, the animals can be asymptomatic or may present nonspecific signs, such as cough, dyspnea, weakness, anorexia, and, in some more serious cases, syncope, caquexia, ascites and death (SEVIMLI et al., 2007; SMITH et al., 2015).

Final diagnosis requires the detection of circulating antigens from adult females or microfilariae in blood samples by microscopic analysis and/or molecular tests (DA SILVA; LANGONI, 2009; TAYLOR et al., 2010).

The infection is cosmopolitan and endemic in tropical and subtropical coastal areas (TAYLOR et al., 2010), with a prevalence of 14% in Rio de Janeiro (RJ) (LABARTHE et al., 1998), 10.7% in Recife (PE) (ALVES et al., 1999), 15% in the state of Santa Catarina (ARAUJO et al., 2003), and 8% in the state of São Paulo (SOUZA; LARSSON, 2001). According to Barbosa, and Alves (2006), the average national prevalence in dogs is approximately 10.2% of microilaric dogs and 9.1% of serologically positive dogs. However, there are no reports of *D. immitis*, in the state of Mato Grosso do Sul (MS). Therefore, the present work aims to report the first case of canine dirofilariosis in the region.

## CASUISTICS

On September 6, 2018, a 14-year-old canine female of the Poodle breed was treated at a private veterinary clinic in Campo Grande, MS (19°55'04 "S and 54°21'50" W), complaining of a nodule in her eye. The animal was castrated, fed once a day with feed, vaccinated and vermifugated annually, had no access to the street and no ectoparasites at the time of care.

Clinical examination showed the presence of a nodule on the left lower eyelid (1 cm in diameter), capillary perfusion time (CPT) of 2 seconds, rectal temperature of 38.5°C, normal heart rate (HR) and respiratory rate (RR), and increased systolic blood pressure (SBP = 180 mmHg) and diastolic blood pressure (DBP = 100 mmHg).

A complete blood count and serum biochemical profile were requested with alanine aminotransferase (ALT), creatinine, alkaline phosphatase (AF), urea, total proteins and globulin. A change was observed only in the blood count, where the sample presented eosinophilia (1,425/mm<sup>3</sup> [Reference: 150- 1250 mm<sup>3</sup>]). Dipyrone 500mg was prescribed, 30mg/kg every 12 hours for

seven days and Anlodipine 0.2 mg/kg, VO, every 12 hours for seven days to control hypertension.

Nine days later (09/15/2018), the patient returned presenting worsening of the clinical condition. The animal presented emesis, melena, tachycardia (135 bpm) and tachypnea (65 rpm). It was requested the admission of the animal and new laboratory tests, such as urinalysis, echocardiogram, urine protein/creatinine ratio (UPC), complete blood count, as well as serum biochemicals, such as alanine aminotransferase (ALT), creatinine, urea, total protein, globulin, cholesterol, lipase, amylase, calcium, phosphorus, alkaline phosphatase (AP), aspartate aminotransferase (AST), gammaglutamyltransferase (GGT), triglycerides, and direct and indirect bilirubins.

On the echocardiogram, right ventricular dilatation, slight left ventricular hypertrophy and slight mitral valve insufficiency were observed. Urinalysis presented intense proteinuria (++++) and increased PCR (0.9 mg/dL [Reference: PCR > 0.5 mg/dL]). In the biochemical examination, an increase in the enzymes ALT (142 U/L [Reference: 10-88 U/L]), AP (191.6 U/L [Reference: 10-92 U/L]), urea (61.76 mg/dL [Reference: 15-40 mg/dL]), amylase (3. 689 U/L [Reference: 300-1,530 U/L]), cholesterol (425.98 mg/dL [Reference: 100-275 mg/dL]), and lipase (1,979 UI/L [Reference: 15-250 U/L]). In the blood count, marked hemolysis had been observed in the plasma, in addition to schistocytes of microfilarias in the blood smear.

An aliquot of the blood sample was forwarded for the morphological and molecular identification of the microfilaria. By the modified *Knott* test, ten microfilaria were evaluated and measured, with a mean length of 312 ± 14 µm and width of 6.2 ± 0, 5 µm, with conical front end and straight back end, identified as *D. immitis* (Figure 1), according to the descriptions of Ramos et al. The diagnosis was confirmed by the SNAP 4Dx Plus® test (IDEXX Laboratories, USA), in which the blood sample was reagent for the detection of circulating antigens from adult females of *D. immitis* and negative for the other pathogens (*Ehrlichia sp.*, *Anaplasma sp.*, and *Borrelia sp.*).

For confirmation, the molecular characterization of the species was performed by the Polymerase Chain Reaction (PCR) technique. The genomic DNA of the blood sample was extracted using the protocol described by Araújo et al. (2009). Then PCR of the 12S rRNA gene was performed according to Otranto et al. (2011). The amplification product (330pb) was visualized after agarose gel electrophoresis (2%) and purified using ExoSAP-IT (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. The sample was sequenced using the 3130-ABI automated sequencer (Applied Biosystems, USA). The chromatograms were evaluated and edited using Contig editor software (Gene Studio) v.2.2.0, and the consensus sequence (284pb) was submitted to BLASTn analysis, presenting 100% identity with 12S rRNA sequences of *D. immitis*. The DNA

sequence obtained in this study was deposited in the Genbank database under the access number MN947412.

Because Campo Grande - MS has no report of dirofilariasis, the owner of the animal was questioned and revealed a history of traveling with the animal to the coastal region in January 2018. During hospitalization Silimarin 50mg/kg was prescribed every 24 hours,

Bezafibrate 2.5 mg/kg every 12 hours, Omeprazole 1mg/kg every 24 hours, Enalapril 0.5m/kg every 12 hours, Doxycycline 10mg/kg every 12 hours; Advocate® (Imidacloprid 100 mg, moxidectin 25 mg) 1mL, single dose. On the fifth day of hospitalization, the animal presented loss of consciousness, severe bradycardia, absence of reflexes, cardiac arrest, and death. The necropsy was not authorized.

Figure 1. *Dirofilaria immitis* in canine blood sample detected by modified Knott's test (400x magnification).



## DISCUSSION

Although *D. immitis* is cosmopolitan and the neighboring states have already reported the occurrence of this parasite (FERNANDES et al., 1999; SOUZA; LARSSON, 2001), this is the first case of canine dirofilariasis in the state of Mato Grosso do Sul.

Confirmation of the diagnosis raised the question whether this case was autochthonous. Nevertheless, the owner reported that the animal traveled to Macaé, RJ, in January 2018, and no preventive measures had been taken. By the fact that the state of Rio de Janeiro is endemic (LABARTHE et al., 1998) and the month in which *D. immitis* microfilariae were detected fit perfectly with the pre-patent period of the disease (DA SILVA; LANGONI, 2009), it is assumed, epidemiologically, that this report represents an allochthonous case.

According to DA SILVA; LANGONI (2009), the introduction and dissemination of helminth to previously free areas, is associated with the circulation of infected domestic dogs and the presence of the vector. Although it is not an autochthonous case, Campo Grande, MS, also has characteristics that can favor the introduction and permanence of the agent, such as climate, deforestation, lack of basic sanitation, and a high population of potential vectors in urban and peri-urban

environments, such as *Aedes aegypti* (*A. aegypti*) and *Culex quinquefasciatus* (*C. quinquefasciatus*).

Recently, a case of microfilaremia by another filarid, *Cercopithifilaria baina* (*C. baina*), had also been reported in this city (TUTIJA et al., 2020). However, as with *D. immitis* and *Acanthocheilonema reconditum* (*A. reconditum*), the clinical and epidemiological importance of these filarides in the region is still unclear. Unlike *D. immitis*, these other filarides are not usually related to serious pathological conditions. However, they need to be differentiated, either by microscopic, serological or molecular analysis, so as not to lead to errors in diagnosis and to allow appropriate treatment of the affected animals (TUTIJA et al., 2020).

The clinical changes associated with dirofilariasis are results of vascular lesions caused by the presence of parasites in the circulatory system of the animal. In most cases, the disease is asymptomatic or shows non-specific signs (SEVIMLI et al., 2007; SMITH et al., 2015). On September 6, 2008, the clinical and laboratory findings did not allow the early recognition of the disease, leading to a compromised prognosis.

Although eosinophilia is present in some cases related to dirofilariasis (SMITH et al., 2015; MONOBE et al., 2016), this hematological finding is inconsistent, as less than half of the dogs with dirofilariasis present it, and it is

commonly found in other infections caused by nematode parasites (MONOBE et al., 2016). This alteration was present in the first blood count, but it was not maintained in the exams of September 15, 2008, even with the intensification of the clinical condition.

In cases of massive infections, the development of circulatory conditions and immune disorders is common. Obstruction of blood flow by right heart failure leads to the deposition of immunocomplexes and fibrin in the glomeruli, which can lead to glomerulonephritis and renal failure (TAYLOR et al., 2010; MONOBE et al., 2016). The echocardiographic findings, intense proteinuria (+++), azotemia, and increased UPC were compatible for the conditions reported above.

Laboratory findings with increased ALT and AP in the present report are suggestive of hepatopathy, corroborating the findings of Taylor et al. (2010) who stated that right congestive heart failure also leads to congestion and injury of the liver, and consequently, to increased hepatic enzyme activity. Pancreatic changes, given the increase in amylase, lipase and cholesterol enzymes, were also observed. These alterations are not usually present, but may be present in more severe cases of dirofilariasis.

Considering the severity of injuries, based on the clinical and laboratory changes observed, the combination of preventive drug Advocate® and doxycyclin was a strongly recommended conduct by the American Heartworm Society before adulticide therapy (Melarsormina) for patients in more severe stages, in order to avoid mass death of adult parasites and consequent vessel obstruction (MONOBE et al., 2016). Before starting the adulticide therapy the animal came to death.

According to Monobe et al. (2016), underdiagnosis is the biggest problem for this infection, especially for animals that do not receive prophylactic treatment and/or are immunosuppressed. These cases are also associated with reduced survival time, resulting in rapid death of the animal due to heart failure.

Therefore, this manuscript is the first report of canine dirofilariasis in Campo Grande, MS, whose immune response was ineffective, with mild clinical and non-specific, and rapid progression, resulting in cardiac, hepatic, renal and pancreatic pathological changes, and probable aloctone due to the displacement of the animal to an endemic region and without prophylactic treatment.

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