Microfilaruria by *Dirofilaria immitis* in a dog: a rare clinical pathological finding

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Abstract A 6-year-old Labrador with history of hyperpernea, respiratory distress, ascites and paraparesis, presented *Dirofilaria immitis* microfilariae in the blood smear, cytology of the abdominal fluid and urine. The positive samples were confirmed by multiplex-PCR (COI gene) and sequencing, and presented 98% homology to *D. immitis* (GenBank accession nos. KC107805.1, EU159111.1, EU163945.1, DQ358815.1, and AJ537512-1). Based on the presented results and the physiopathology of the heartworm disease, molecular techniques may be helpful and accurate in the diagnosis of the disease in vertebrates, and microfilaruria, even uncommon, should be considered on a renal failure evaluation.

Keywords Microfilaruria · Molecular techniques · Heartworm disease · Dog

Introduction

Heartworm disease is a worldwide mosquito-borne disease caused by a filarial nematode, *Dirofilaria immitis*. It occurs in tropical, subtropical, and some temperate regions, especially in North America. In Brazil, the prevalence is associated to the coastal areas and the presence of mosquitoes, i.e., *Aedes* spp., *Anopheles* spp. and *Culex* spp. (Acha and Szyfres 2003; Fernandes et al. 2000). Moreover, favorable climatic conditions, traffic among disease-free, silent and endemic regions, as well as density of human population and the presence of other canid populations also contribute to this prevalence. The adult worms live in the right ventricle of the heart and adjacent blood vessels. The infective-stage larvae develop at the infection site, and migrate to the right heart for the maturity. The adult parasite produces microfilariae, which circulate into the peripheral blood (Acha and Szyfres 2003; Da Silva and Langoni 2009).

The clinical signs vary widely from asymptomatic to congestive right heart failure, and acute or chronic vena cava syndrome. Depending on the adult worm burden, host-pathogen response, the duration of infection, and level of exercise, the disease can be life-threatening to the infected dog (Da Silva and Langoni 2009; Venco et al. 2005). The diagnosis is usually based on the morphological identification of the circulating microfilariae, and immunodiagnostic tests, i.e., enzyme-linked immunosorbent assay (ELISA). The use of molecular techniques, i.e., polymerase chain reaction (PCR), is still uncommon for the diagnostic routine of this disease. Even that, it has been considered a powerful tool on helping the differential diagnosis when required.

Although microfilaruria can occur, only few cases have been published reporting this rare finding (Kaewthamasorn

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et al. 2008). In this way, this study reports the first case of microfilaruria by *D. immitis* in a dog from Brazil, confirmed by molecular techniques.

Case presentation

A 6-year-old, intact female dog, Labrador, 46 kg, from São Paulo, SP, Brazil (23°32'50"S; 46°38'09"W), was presented with a 2-days history of hyperpnea, respiratory distress, moderate dehydration, anorexia, ascites and paraparesis at the Veterinary Hospital of São Paulo State University, Brazil. The dog had been vaccinated annually but had never been challenged to any prophylactic treatment for dirofilariasis. On physical examination, the animal presented lateral decumbency, tachypnea (60 breaths min^{-1}), tachycardia (150 beats min^{-1}), heart murmurs and moderate muffled heart sounds. Pale mucous membrane and temperature of 39.5 °C was also reported. A complete blood cell count (CBC) showed intense non-regenerative anemia, mild leukocytosis by neutrophilia, eosinophilia and monocytosis, and mild thrombocytopenia. Leukocyte differentiation and thrombocytopenia were confirmed on both blood smear examination and CBC panel. High number of microfilariae was observed in the blood smear (3–5 filaria field $^{-1}$, 10 $\times$ objective) (Fig. 1). The serum biochemical analysis revealed a mild azotemia, increased alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), hypoalbuminemia and hypoproteinemia. Thoracic radiographs were taken for suspected cardiogenic ascites. The radiographic findings included pleural effusion and cardiac enlargement, followed by dorsal displacement of the trachea and right ventricle dilation. No electrocardiographic alteration was observed other than sinus tachycardia. Around 4 L of abdominal effusion were collected and sent for cytological

analysis. The cytological examination of the abdominal effusion showed a hemorrhagic effusion with high number of leukocytes, mainly lymphocytes and neutrophils, and rare microfilariae. Urine sample was collected by catheterization for analyzes. Urinalysis revealed low density, proteinuria, bilirubinuria, granular and hyaline casts, hematuria (Fig. 2), pyuria, and moderate amount of microfilariae. The analysis of the motility pattern in fresh urine showed a stationary movement of the filarial parasites, typical of *Dirofilaria* spp. (Figs. 2, 3). Blood, urine and abdominal fluid samples were researched for filarial nematode DNA (*D. immitis*, *D. repens*, *Acanthocheilonema* (= *Dipetalonema*) *reconditum*, *Cercothiilaria* spp.) by using a multiplex PCR targeting the mitochondrial cytochrome c oxidase subunit 1 (COI) gene (Latrofa et al. 2012). All samples resulted positive for *D. immitis* by PCR. Then, the PCR products were sequenced for confirmation, and presented 98% homology to *D. immitis* cytochrome oxidase subunit I gene partial cds; mitochondrial (GenBank accession nos. KC107805.1, EU159111.1, EU163945.1, DQ358815.1), and 98% *D. immitis* complete mitochondrial genome (GenBank accession no. AJ537512-1).

The initial therapy consisted in oxygen and fluid therapies, administration of furosemide (2 mg kg^{-1} iv every 6 h) and ampicillin (20 mg. kg^{-1} iv every 12 h). After 24 h, the animal increased the respiratory distress and died. The owner did not authorize necropsy.

Discussion

Dirofilariasis is a zoonotic disease with a high public health importance. It can be caused by *D. immitis*, *D. repens*, *D. tenuis* and *D. ursi* (Acha and Szyfres 2003; Da Silva and Langoni 2009; Pereira et al. 2015), in which the first one is

Fig. 1 Sheathed microfilaria in peripheral blood smear (Panoptic stain, $\times 10$ objective)

Fig. 2 Microfilaria in fresh urine slide of a dog. Arrows represents red blood cells ($\times 10$ objective)

Fig. 3 Microscope visualization of *Dirofilaria immitis* microfilaria in the dog urine ($\times 10$ objective)

the most important for dogs (Acha and Szyfres 2003; Ambily and Usha 2012). It is frequent in all geographical regions from Brazil, mainly in the coastal States. The prevalence can be higher than 60% in endemic foci and as lower as 2% (Dantas-Torres 2008). From 1988 to 2001, the prevalence countrywide has decreased from 7.9 to 2%, probably due to effective prophylactic measures (Labarthe and Guerrero 2005). The reported dog lived in Sao Paulo, SP, where the prevalence is 3.89% (Souza and Larsson 2001). Therefore, the animal had never had prophylactic treatment for dirofilariasis.

The cases in non-endemic areas are often underdiagnosed for this disease. It happens due to microfilaremia not be a common finding in non-endemic areas by directly diagnosis, which culminate with the probably low number of adult parasites. It increases the false-negative results on immunodiagnostic tests, and labels this disease as neglected (Da Silva and Langoni 2009). Underdiagnose is often associated with reduced survival time probably because the maturity of the parasites, which reproduce during the first unspecific clinical signs. It leads to acute heart and renal failures, as observed on this reported case. In addition to the acute heart failure, the animal presented liver congestion in less than 2 days.

Clinical signs are usually associated to the life cycle of nematode. During microfilaremia, the number of adult parasites is low and they are still migrating; so, most of the patients are asymptomatic or have unspecific clinical signs, i.e., persistent cough, anorexia, loss of weight and fever (Polizopoulou et al. 2000). During the adult phase, the parasites locate at the right atrium and ventricle, leading to pulmonary hypertension, myocardial hypertrophy and heart failure. Consequently, the animal develops chronic right-sided congestive heart failure (CHF) and hepatic insufficiency (Kaewthamasorn et al. 2008). It implies to the most observed clinical signs, i.e., exercise intolerance, hyperpnea, respiratory distress, tachycardia, arritmia, heart murmurs and ascites. Respiratory distress and hyperpnea can also be caused by spontaneous thromboembolism during

the death of nematode (Polizopoulou et al. 2000; Venco et al. 2005).

The most observed hematological alterations in dogs with dirofilarioses are mild thrombocytopenia, moderate to intense nonregenerative anemia, mild leukocytosis by neutrophilia, eosinophilia and basophilia, and microfilaremia. Biochemical alterations are also common, mainly those affecting liver and kidneys, i.e., increased creatinine and blood urea nitrogen (BUN), increased ALT, ALP, GGT, bilirubin and hypoalbuminemia (Kaewthamasorn et al. 2008; Venco et al. 2005).

In massive infections, as observed on this report, severe circulatory and immune-mediated disorders are common. The obstruction of blood flow by the parasite leads to congestive right heart failure, which cause abdominal effusion. Moreover, microfilariae may occlude and rupture small vessels. In this way, the microfilariae are released in atypical places, i.e., peritoneal and abdominal effusion and, even, lower urinary tract (Venco et al. 2005). In addition, the immune complex and fibrin deposition in the glomeruli may lead to glomerulonephritis and renal failure.

In fact, the association between acute renal failure and dirofilariasis is often described. Some researchers also observed chronic interstitial nephritis associated with azotemia, polyuria, polydipsia, dehydration and alteration on urinalysis, i.e., low density, proteinuria, bilirubinuria, granular casts and pyuria (Ambily and Usha 2012; Kaewthamasorn et al. 2008; Venco et al. 2005). However, the presence of microfilaria in urine is an unusual finding. Up to now, just two cases had been described and both associated with cystitis. Microfilaruria may occur due to hemorrhagic inflammation of lower urinary tract or by the penetration of the microfilaria into the glomerular capillary, which increase the chances of renal failure (Kaewthamasorn et al. 2008).

The mortality is usually associated with acute heart and renal failures. During the treatment, the mortality is often associated with acute pulmonary thromboembolism (Polizopoulou et al. 2000).

The routinely diagnosis of canine heartworm disease is based on the direct observation of circulating microfilariae and immunodiagnostic tests. However, the morphologic differentiation between *D. immitis* and *D. repens* is usually complicated by the direct observation. Serological tests, although sensitive, are limited to the host immune response. Another drawback is the possibility of cross-reaction with other parasites. Based on that and to the moderate to mild amount of microfilaria on blood, abdominal effusion and urine, all samples submitted and confirmed to be *D. immitis* by molecular techniques.

In the same way, direct diagnosis needs specific personal training for the morphological classification of the parasites on the direct diagnosis. The molecular diagnosis allows

specific, sensitive and accurate results for many infectious diseases and cancers, with high performance. So, the tendency is to use more frequently PCR in routine diagnosis of dirofilariasis, not only as differential tests.

Conclusion

Microfilaruria by *D. immitis* should be considered on a canine renal failure evaluation, and molecular techniques help the confirmation of the pathogen.

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