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Microfilaremia by *Cercopithifilaria bainae* in a dog from the central western region of Brazil: case report

[*Microfilaremia por Cercopithifilaria bainae em um cão da região Centro-Oeste do Brasil: relato de caso*]

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ABSTRACT

Cercopithifilaria bainae is a nematode belonging to the family Onchocercidae that parasitizes the subcutaneous tissue of dogs. Its transmission occurs through the tick *Rhipicephalus sanguineus* and its geographical distribution overlaps that of this vector. The present study reports the detection of microfilaremia by *C. bainae* in an eight-year-old male dog that presented anorexia, hyperthermia, motor incoordination, mydriasis, a nodule in the left testicle and concomitant infection by *Ehrlichia* sp. Blood samples were analyzed using microscopy, PCR and DNA sequencing. Microfilariae measuring 150±5.5µm in length and 7±1.8µm in width were retrieved. The DNA sequence exhibited 98% identity with *C. bainae* sequences available in Genbank. This is the first report of microfilaremia by *C. bainae* in a dog in the central western region of Brazil.

Keywords: dog, microfilariae, Brazil, DNA

RESUMO

Cercopithifilaria bainae *é um nematoide pertencente à família Onchocercidae, que parasita o tecido subcutâneo de cães. Sua transmissão ocorre pelo carrapato* Rhipicephalus sanguineus, *e sua distribuição geográfica se sobrepõe ao espalhamento desse vetor. O presente estudo relata a detecção de microfilaremia por C.* bainae *em um cão macho de oito anos que apresentava anorexia, hipertermia, incoordenação motora, midríase e nódulo no testículo esquerdo e infecção concomitante por* Ehrlichia *sp. A coleta de sangue foi realizada, e o material analisado por meio dos exames de microscopia, PCR e sequenciamento de DNA. Microfilárias medindo 150±5,5µm de comprimento e 7±1,8µm de largura foram recuperadas. A sequência de DNA obtida mostrou 98% de identidade com sequências de C.* bainae *disponíveis no Genbank. Este é o primeiro relato de microfilaremia de C.* bainae *em um cão na região Centro-Oeste do Brasil.*

Palavras-chave: cão, microfilárias, Brasil, DNA

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INTRODUCTION

Filarioids of the family Onchocercidae include species with microfilariae that circulate in the bloodstream (*Dirofilaria immitis* and *Acantocheilonema reconditum*) or parasitize the subcutaneous tissue of vertebrate hosts (*Cercopithifilaria* sp. and *Onchocerca lupi*) (McCall *et al*., 2008). Among these nematodes, those of the genus *Dirofilaria* and *Acantocheilonema* have been extensively studied throughout the world and their pathogenic role is well defined (McCall *et al*., 2008). Conversely, species that inhabit subcutaneous tissue (*Cercopithifilaria* and *Onchocerca*) have been under-investigated.

The genus *Cercopithifilaria* comprises 28 different species of filarioids, three of which (*Cercopithifilaria grassi*, *C. bainae* and *Cercopithifilaria* sp. II sensu) parasitize dogs. Among these species, *C. bainae* is considered the most common in dogs and its biology has been studied in recent years (Otranto *et al*., 2012). The distribution of this species is believed to be strictly associated with that of its main arthropod vector – the tick *Rhipicephalus sanguineus* sensu lato (Brianti *et al*., 2012). The first report of *C. bainae* in dogs was published more than thirty years ago (Almeida and Vicente, 1984), but this nematode has since been largely ignored. In recent years, *C. bainae* has been described in dogs and/or ticks in several countries (Latrofa *et al*., 2014; Solinas *et al*., 2014; Santos *et al*., 2017).

C. bainae has been considered a filarioid of minor pathogenic relevance. However, dermatological conditions, such as perivascular interstitial dermatitis, have been associated with infection by this parasite (Otranto *et al*., 2012). Moreover, a case of chronic polyarthritis has been described in a European dog (Gabrielli *et al*., 2014).

In Brazil, information on the occurrence of *C. bainae* is available in dogs and *Rhipicephalus sanguineus* only for the southeastern (Almeida and Vicente, 1984) and northeastern regions (Latrofa *et al*., 2014; Ramos *et al*., 2016), however there is no information of this filarioids in other regions of the country, despite the wide distribution of the tick vector. This paper reports a case of *C. bainae* in a dog in the central western region of Brazil (state of Mato Grosso do Sul) showing atypical microfilaremia.

CASUISTRY

In September 2016, an eight-year-old male poodle in the municipality of Bandeirantes (19º55'04" S and 54º21'50" W) in the state of Mato Grosso do Sul (central western region of Brazil) was admitted to a private veterinary practice with the complaint of vomiting and diarrhea. The clinical examination revealed hyperthermia, motor incoordination, mydriasis, anorexia and an increase in left testicular volume. Blood sampling was performed for the blood cell count. The hematological analysis revealed hypochromic normocytic anemia, neutrophilia, eosinophilia, lymphopenia and thrombocytopenia. Other findings included morulae of *Ehrlichia* sp. and microfilaria in the blood with a mean size of 150.42±5.5µm in length and 7.09±1.8µm in width (Figure 1).

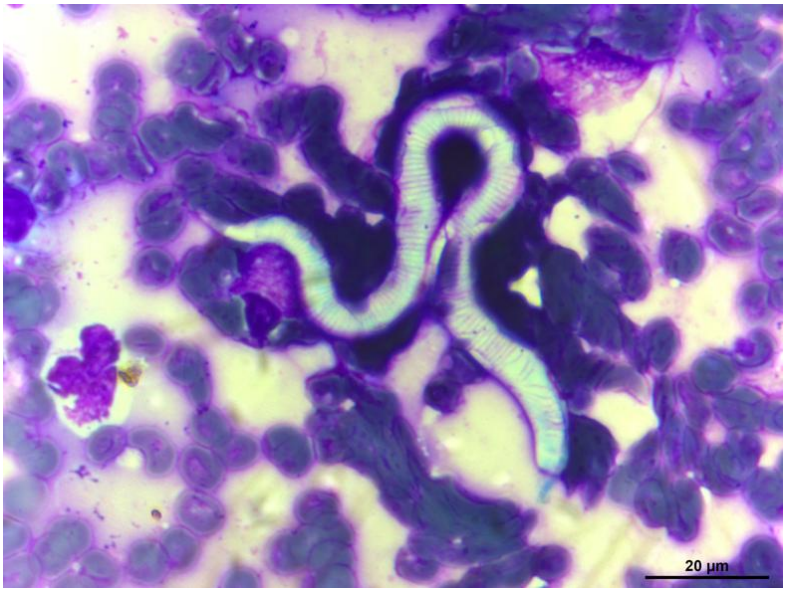

Figure 1. Microfilaria in blood sample from dog.

The animal was treated with febantel 15mg/kg, pyrantel 14.4mg/kg, praziquantel 5mg/kg, ivermectin 0.006mg/kg (Endogard® - Virbac) and doxycycline 10mg/kg and was kept under observation, but died after five days due to clinical complications.

At necropsy, a marked increase in the volume of both testicles was observed. The right testicle exhibited diffuse suppurative necrotizing orchitis of an undetermined origin and a diagnosis of seminoma was made in the left testicle. In the liver, plasma extravasation was found, with inflammatory infiltrate of neutrophils and plasma cells, fibrinoid necrosis in the paracentral region and fibrillar eosinophilic material partially occluding the blood vessel lumen. In the kidneys, we found increased cellularity in the glomerular base, predominantly inflammatory infiltrate of lymphocytes and peri-glomerular plasma cells, and interstitial foci adjacent to the renal pelvis. In the spleen, we found multiple foci of fibrinoid necrosis in the white pulp, abundant fibrillar eosinophilic material interspersed with lymphocytes, plasma cells and macrophage populations, which were attributed to infection by *Ehrlichia* sp.

For the identification of the microfilaria, genomic DNA was extracted from the blood sample following protocol described by Araújo *et al.* (2009). The integrity and quantity of the DNA was evaluated by 1% agarose gel electrophoresis and spectrophotometry (A260/A280), respectively.

A PCR was carried out using the primer set Fila12SF (5'-CGGGAGTAAAGTTTTGTTTAAACCG-3') and Fila12SR (5' -CATTGACGGATGGTTTGTACCAC-3') (Otranto *et al*., 2012) developed to amplify a 330-bp fragment of 12S rRNA gene common to filarioids of the genera *Acanthocheilonema*, *Cercopithifilaria* and *Dirofilaria*. The PCR assay was performed in a final volume of 25µL containing 10mM tris-HCL (pH 8.3), 50mM of KCl, 1.5mM of $MgCl_2$, 0.2mM of each deoxynucleotide (dNTP), 1.25U of Taq DNA polymerase (Invitrogen), 10pmol of each primer, approximately 100ng of genomic DNA and H_2O to a final volume of 25uL. The thermocycling conditions were 80°C for one minute, 95°C for five minutes, followed by 40 cycles at 94°C for one minute, annealing at 58°C for one minute and extension at 72°C for one minute. A final extension step was performed at 72°C for one minute.

The amplification products were viewed under an ultraviolet light after electrophoresis on 2% agarose gel stained with GelRed® Biotium) following the manufacturer's instructions. DNA from *C. bainae* (characterized by Ramos *et al*., 2016) and nuclease-free water were used at the positive and negative control reactions, respectively.

The amplicon (approximately 330pb) was purified with CleanSweep PCR Purification Reagent (Thermo Fisher Scientific) following the manufacturer's instructions and submitted to DNA sequencing in both directions using the Sanger method in an ABI-3130 automatic sequencer (Applied Biosystems). The chromatograms were evaluated and edited using the Contig Editor program (Gene Studio) v. 2.2.0 and the consensus sequence (272bp) was submitted to BLASTn analysis. Sequences of *C. bainae* showed 98% homology to those available in GenBank database (accession numbers KX156956, MG793438 and MG793436). The consensus DNA sequence obtained in the present study was deposited in Genbank under accession number MH972532.

DISCUSSION

Among the filarioids that infect dogs, those transmitted by culicid vectors (*Dirofilaria immitis* and *Dirofilaria repens*) have been extensively studied (McCall *et al*., 2008). Attention has recently been given to species of the genus *Cercopithifilaria*, the microfilariae of which can be detected under the skin of dogs, but little is known regarding its pathogenic potential (Santos *et al.*, 2017).

Due to the wide distribution of its vector *R. sanguineus* (Brianti *et al*., 2012), the distribution of filarioids is also extensive (Latrofa *et al*., 2014; Solinas *et al*., 2014; Ramos *et al*., 2016), but the prevalence in dogs varies. Ramos *et al*. (2016) found a rate of only 0.96% (1/104) among animals in northeastern Brazil. In contrast, Solinas *et al*. (2014) found a rate of 9.4% (17/180) in dogs in Italy. The low prevalence of the filarioids in some regions may account for the lack of veterinarian knowledge and little clinical importance attributed to the nematode, making it difficult to obtain information on its actual pathogenic potential.

In Brazil, *R. sanguineus* sensu lato is the most prevalent tick species in dogs and is responsible for the transmission of several pathogenic microorganisms, such as *Ehrlichia canis, Babesia canis vogeli, Hepatozoon canis* and even *C. bainae* (Santos *et al*., 2017). In the present report, although no ticks were found during the clinical examination, the transmission of the filarioid to the dog likely occurred through this route, since morulae from *Ehrlichia* sp. were found during the hematological analysis. This pathogen is common among dogs in the region and is known to be transmitted by *R. sanguineus* (Soares *et al*., 2017).

Cercopithifilaria sp., (Ie. *C. bainae* and *C. grassii*) was reported in *R. sanguineus* sensu lato by Latrofa *et al.* (2014) in 9,6% (17/177) from several country's samples, including three positive in Pernambuco- Brazil, Santos *et al.* (2017) founded *C. bainae* in 2.68% (51/1906) of dissected ticks at the same region. However, there are no studies of this filarioids in *R. sanguineus* in other regions of Brazil, despite the wide distribution of this vector.

Adult nematodes of *C. bainae* as well as their larvae (microfilariae) are found in the subcutaneous tissue and are difficult to detect, unlike *D. immitis* and *A. reconditum*, which are found in the blood (Ramos *et al*., 2016). The dog in the present report had microfilariae in the blood, which may be considered an ectopic location, based on the information currently available on the lifecycle of the pathogen described by Brianti *et al*. (2012). Other aberrant locations of *C. bainae* microfilariae have been reported in dogs, such as the synovial fluid (Gabrielli *et al*., 2014). The ectopic location of other members of the family Onchocercidae has also been reported, such as *Onchocerca lupi* found in the larynx of a dog (Alho *et al*., 2016).

The differentiation between types of filariasis is important for the prescription of the treatment and the establishment of a prognosis. *D. immitis* leads to severe heart disease and requires expensive, potentially aggressive therapy (McCall *et al*., 2008). In contrast, infection by *C. bainae* is usually an accidental finding, with no clinical signs (Otranto *et al*., 2012). The animal in the present report presented microfilaremia, which occurs with *A. reconditum* and *D. immitis* and could therefore have led to improper treatment.

The microfilariae species can be differentiated by morphological observation. Studies by Ramos *et al.* (2016) show that *D. immitis* has 301.2±7.6µm long and 5.9±0.8µm wide on average and features a conical front end and a straight posterior end. Microfilariae of *A. reconditum* are 272.3±4.3µm in length and 4.1±0.3µm in width,

and are characterized by a blunt front end. Conversely, microfilariae of *C. bainae* has 180.2±2.3µm in length and 4.6±0.2µm in width rounded head, short dorsal-ventrally flattened body and thick cuticle presenting transverse striations. The morphometric analysis of the microfilariae found in this study revealed measures somewhat lower than those reported by Ramos *et al*. (2016). These variations may be attributed to differences in the stage of larval development. Another possible explanation would be genetic variation among nematode strains.

Due to the complexity of the clinical condition found in the dog of the present report, it is difficult to establish the actual pathological contribution of *C. bainae*. However, as observed in other studies, the ectopic location of the microfilaria likely triggered a local immune response capable of causing damage to adjacent tissues. Thus, it is essential to conduct studies involving the experimental infection of animal models to clarify the pathological potential of this filarioid.

This report is the first description of *C. bainae* in a dog from central western Brazil. The atypical case of microfilaremia underscores the importance of considering this aspect in the differential diagnosis in order to avoid misdiagnosis and inadequate treatment.

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