

*Case report*

**ACANTHOICHEILONEMA RECONDITUM (SPIRURIDA: ONCHOCERCIDAE) IN THE ORAL CAVITY OF A DOG IN NORTHEASTERN BRAZIL: CASE REPORT**

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An 11-year-old male dog presenting exophthalmia and perforation of the cornea of the left eye was seen at the veterinary hospital of the State University of Maranhão. Upon physical examination, irregularly shaped granulomas of crumbly appearance were observed on the dog's tongue. The dog was sent for ocular enucleation surgery. The following tests were requested: complete blood count, serum biochemistry, hemoparasite investigation, chest X-Ray, echocardiogram, electrocardiogram, cytology and nodule histopathology. The blood count revealed neutrophilia, signs of anemia, increased total plasma protein and thrombocytopenia. Serum biochemistry and the imaging examinations showed normal results. The cytological examination on the tongue nodules showed microfilariae and the histopathological examination showed chronic glossitis. Polymerase chain reaction (PCR) and genetic sequencing were performed on a blood sample, which made it possible to identify the species *Acanthocheilonema reconditum*. This is the first record worldwide of the occurrence of microfilariae of *A. reconditum* on tongue nodules. This finding serves to alert dermatologists and dentists regarding the need for a differential diagnosis for types of oral cavity lesions of unknown etiological origin.

**Keywords:** microfilaria, tongue, dog, *Acanthocheilonema reconditum*

## INTRODUCTION

Canine filariasis is a vector-borne parasitic disease caused by various filarial nematodes belonging to the families Filariidae and Onchocercidae [1]. In dogs, this disease has a cosmopolitan distribution, but it occurs mainly in tropical regions given the higher concentration and diversity of vectors in the tropics [2]. Some species in these families are considered to present little pathogenicity, but others may even cause death and present a zoonotic potential [3-5].

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In Brazil, the filarial species *Acanthocheiloneema reconditum* (syn = *Dipetalonema reconditum*), *Cercoptifilaria bainaie*, *Dipetalonema grassi*, *Dirofilaria immitis* and *Dirofilaria repens* have been recorded parasitizing wild and domestic canids [6]. However, like in other regions of the world, *A. reconditum* and *D. immitis* are the most prevalent species in Brazil [7,6].

*A. reconditum* is the etiological agent for dipetalonemiasis. It inhabits the subcutaneous tissues, perirenal region and peritoneal cavity of canids and is transmitted by common ectoparasites of cats and dogs (fleas, lice and ticks) [2,8,9]. Although this disease does not have any severe pathological consequences for its hosts, it causes a transitory infection, with cutaneous ulcerations and subcutaneous abscesses [9]. *A. reconditum* has public health importance, given that cases of human infection have been documented [10,11].

In Brazil, the range of *A. reconditum* covers much of the country but until now there had not been any records of its occurrence in the state of Maranhão, in northeastern Brazil. Worldwide, until now, there had not been any records of locating this parasite in the oral cavity of dogs. The objective of this report was to register the occurrence of *A. reconditum* on tongue nodules in a dog, which was diagnosed through cytological and molecular examinations.

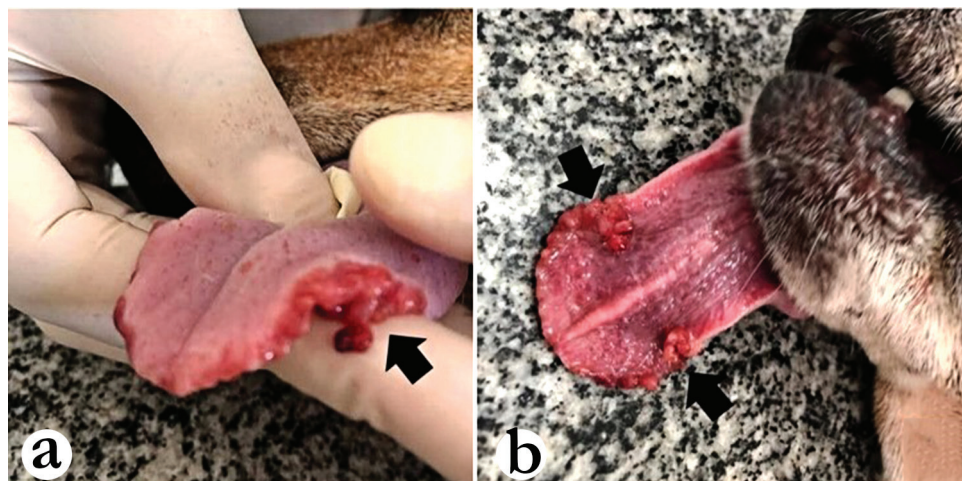
## CASE REPORT

An 11-year-old male dog of the breed Fox Paulistinha (Brazilian terrier), presenting exophthalmia and perforation of the cornea of the left eye, was seen at the veterinary hospital of the State University of Maranhão, in São Luís, Maranhão, Brazil. On physical examination, the dog was seen to be experiencing discomfort because of the lesion. The dog's mucosa presented normal coloration, palpable lymph nodes were normal, rectal temperature 36.8 °C, heart rate 100 bpm, respiratory rate 70 movements per minute (mpm) and ectoparasites absent. Its general clinical state was stable, without any history of other diseases, and its vaccination and deworming schedules were up to data. In examining the oral cavity, irregularly shaped granulomas of reddish color and crumbly consistency were observed in the mucosa of the tongue (Figure 1).

As an initial measure, the dog was sent to the surgical center for enucleation of the left eyeball. The following preoperative laboratory tests were requested: complete blood count, serum biochemistry (aspartate aminotransferase [AST], alanine aminotransferase [ALT], urea, creatinine, alkaline phosphatase and albumin), investigation of hemoparasites (*Babesia* sp. and *Ehrlichia* sp.), chest X-Ray, echocardiogram and electrocardiogram. Cytological examination of the nodules and histopathological examination of the tongue were also requested with the aim of clarifying the origin of the nodules.

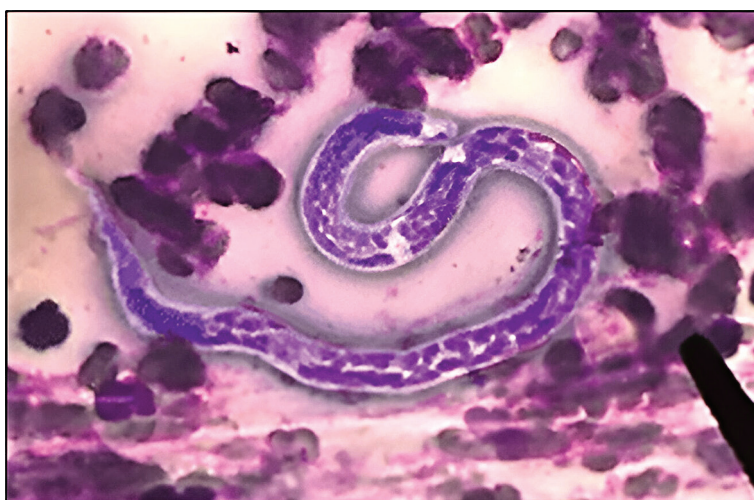
The hematological alterations revealed leukocytosis ( $20,000 \times 10^3/\mu\text{l}$ ) due to neutrophilia ( $15,600 \times 10^3/\mu\text{l}$ ; IR:  $3,000-11,500 \times 10^3/\mu\text{l}$ ); signs of anemia with low red cell count ( $4.17 \times 10^6/\mu\text{l}$ ), low hematocrit (25%); increased total plasma protein

(11.2 g/dl) and thrombocytopenia ( $170,000/\text{mm}^3$ ). No alterations were seen in serum biochemistry tests or on imaging examinations.



**Figure 1.** Granulomas in the mucosa of the tongue a dog (arrows).  
a) Lateral view; b) Ventral view

On cytological examination of the tongue nodules, a moderate quantity of neutrophils, rare macrophages and a small number of eosinophils were observed. Among these, there were structures compatible with microfilariae, characterized by a rounded frontal edge and hook-shaped posterior extremity, which was suggestive of microfilariae *A. reconditum* (Figure 2). After the microfilariae were seen in the cytological examination



**Figure 2.** Microfilariae in the cytological examination of the nodules in the tongue of a dog. Panoptic stain. 100x.

of the nodules, blood samples were collected to perform the thick-drop test [12] and Knott test [13], which were both negative, and to make a molecular diagnosis.

The histopathological examination on tongue tissue showed epithelial hyperplasia, areas of necrosis, hydropic degeneration of the epithelium and deposition of eosinophilic material and fibrin. In the lamina propria, a diffuse inflammatory process composed of foamy macrophages and plasmacytes was observed, thus characterizing chronic glossitis. No parasites were observed on the histopathological examination.

With the aim of achieving molecular confirmation of the species *A. reconditum*, DNA was extracted from a blood sample by means of the commercial kit InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA), in accordance with the manufacturer's recommendations. Following this, the material was subjected to the polymerase chain reaction (PCR), using the oligonucleotides Fila12SF: 5'-CGG GAG TAA AGT TTT GTT TAA ACC G-3' and Fila12SR: 5'-CAT TGA CGG ATG GTT TGT ACC AC-3', which amplify fragments of the gene 12S rDNA of filariids [14]. The sample was found to be positive through PCR and, after genetic sequencing and comparison with sequences available from GenBank, it presented 100% similarity to *A. reconditum* (access number: JF461460). The dog subsequently died because of other causes, and thus treatment of the parasitic disease became impossible.

## DISCUSSION

Among the filariid nematodes diagnosed in dogs within veterinary practice, *A. reconditum* is the least pathogenic. Cutaneous presence of this parasite may be manifested in the form of small subcutaneous nodules, which on rare occasions may be painful or pruriginous. Adult stages and microfilariae are found inside these nodules [15].

At the time when this dog was seen at the veterinary hospital, it was in a clinically stable condition, without any indications of disease. This was in line with most reports on *A. reconditum* in dogs: no occurrences of illness associated with confirmed cases of infection have been described [2,8]. Nevertheless, when infection of great severity is present, animals may present pruritus, alopecia and dermal lesions that sometimes are suppurative [16]. According to Hoseini et al. [17], many of the symptoms attributed to *A. reconditum* may only be manifestations of parasitism concurrent with other pathogens.

The abnormalities found in the hematological tests (leukocytosis due to neutrophilia, signs of anemia, increased plasma protein and thrombocytopenia) were consistent with the findings from previous studies on natural and experimental infection by *A. reconditum* in dogs [18,19]. In a study conducted by Espinosa et al. [20], cases of canine filariasis due to *A. reconditum* were statistically associated with dogs that presented clinical signs of anemia, low hemoglobin and hematocrit levels and high plasma protein levels, similar to what was found in the present case.

It is important to emphasize that the increased neutrophil count observed here may not have been directly associated with the infection by *A. reconditum*. The fact that this dog was under stress because of ocular discomfort needs to be taken into consideration. Stress generally results in increased levels of the components of leukocyte counts, especially neutrophils [21].

In the biochemical assessment, the dog did not present any indications of hepatic or renal lesions. This was in line with what was seen in the majority of the dogs sampled in the study by Pacifico et al. [19]. In contrast, Hashem and Badawy [18] reported that there were significant increases in the serum levels of AST, ALT, bilirubin, total proteins and globulins and decreased glycemia in dogs with *A. reconditum*, compared with uninfected dogs.

The histopathological evaluation on tongue tissue showed the presence of epithelial hyperplasia, areas of necrosis, hydropic degeneration of the epithelium and deposition of eosinophilic material and fibrin, along with the presence of foamy macrophages and plasmacytes, which characterize chronic glossitis. These findings indicate the existence of a reaction to the microfilariae of the parasite, which was observed in the cytological examination. In fact, in cases of chronic inflammation resulting from infection, lingual papillae increase in volume, thus giving the tongue a granular appearance [22]. Allied with this, the microfilariae may also be free-living in the dermis or subcutaneous region, or in nodules [23], as was observed in the present report.

The case described in the present report can be considered to be the first record so far of natural infection by *A. reconditum* on the tongue of a dog. Oral filariasis has only previously been reported in humans, and in those cases adults and microfilariae were observed in submucosal nodules under the oral mucosa, mainly involving species of *Dirofilaria* [24,25].

There are no studies addressing possible mechanisms for survival of the microfilarial and adult phases of *A. reconditum* in host tissues, in the literature available. The scarcity of such information makes it difficult to explain the finding of this parasite in the nodules in the tongue mucosa. One event observed on the histological slides of the present study was the presence of an eosinophilic infiltrate, which was also described in previous reports [16,23]. This was probably related to the local immune and anti-allergic response triggered by the presence of microfilariae. Moreover, perhaps this explains the survival of the parasite on the tongue.

Corroborating the above hypothesis, experimental studies on other nematodes that inhabit tissues have suggested that eosinophils support the growth and survival of the parasite in the tissue, through regulation of local immunity [26,27]. In an experiment conducted by Babayan et al. [28] on *Litomosoides sigmodontis* (family Onchocercidae, i.e. the same family as *A. reconditum*) in mice, it was concluded that eosinophilia was a primary host-determined factor regarding the life expectancy of filariids, both in the larval and in the adult phase. In addition, the capacity of filaria to adjust their

development and reproduction over time, in relation to regulation of the local immune response mediated by eosinophils, was demonstrated in that report [28].

The detection of microfilariae in the cytological aspirate from nodules on the dog's tongue led to suspecting the presence of *A. reconditum*. Its circulating microfilariae are similar to those of *D. immitis* and may be confounded with the latter when observed through light microscopy in routine laboratory diagnoses. However, while the microfilariae of *A. reconditum* have a thinner and shorter body with a cephalic hook and a hook-shaped curved tail, the microfilariae of *D. immitis* do not have a cephalic hook [2]. Careful morphological examination of the microfilariae needs to be undertaken in order to differentiate these parasites, but this morphological identification is not always achieved, mainly because the analyst may be inexperienced. In view of this possible limitation, DNA extraction and genetic sequencing enable identification of the species *A. reconditum*.

No microfilariae were observed during the thick-drop and Knott tests. This may have been due to low levels of microfilaremia, which can be correlated with the course of the infection. In fact, experimental studies have indicated that microfilaremia tends to diminish over time and is also affected by the fertility of females of this parasite [8]. Differing from what was seen in the present case, Engelmann et al. [16] observed presence of microfilariae of *A. reconditum* both in the bloodstream and in cytological examination of a thoracic nodule from a female dog.

In Brazil, studies have documented morphological and molecular identification of *A. reconditum* in the bloodstream, mainly in coastal regions [29-31], but also in non-coastal areas [32]. There have also been rare findings of the parasite in subcutaneous tissues [16,33].

Nonetheless, it is likely that *A. reconditum* is more widely disseminated in Brazil. The following factors may have contributed to this: lack of clinical importance for this parasite; erroneous identification of microfilariae in parasitological tests; or even non-publication of cases of *A. reconditum* diagnosed by veterinarians and in clinical laboratories.

The present report expands the geographical distribution of *A. reconditum* in Brazil. The case described here was the second one documented in the Amazon region [29], but the first in the state of Maranhão. This state is located at the intersection of three biomes (Cerrado, Amazon and Caatinga) and has great diversity of climates and ecosystems, along with high occurrence of deforestation and silt transportation in rivers [34,35]. These characteristics favor biological development of the vectors for filariids and, together with the disorderly growth of the population of stray dogs, enable transmission of the parasite between animals. In a study by Costa et al. [36], ectoparasites on dogs in urban and rural areas of Maranhão were investigated. Abundant occurrence of three of the arthropod species that have been incriminated in transmission of *A. reconditum* was recorded: the tick *Rhipicephalus sanguineus* (78.0%), the flea *Ctenocephalides felis* (16.0%) and the louse *Heterodoxus spiniger* (2.0%).

The occurrence of microfilariae of *A. reconditum* in tongue nodules reported here is the first record of this worldwide. This finding serves to alert dermatologists and dentists regarding the need for a differential diagnosis for types of oral cavity lesions of unknown etiological origin. Furthermore, it is important for veterinary pathologists to become familiar with the characteristics of this filariid in routine histopathological examinations on cutaneous nodules. Given that the treatment for *A. reconditum* differs from what is used for *D. immitis*, it is essential to seek to correctly differentiate these species.

### **Authors' contributions**

TFC has been involved in drafting the manuscript or revising it critically for important intellectual content. SAB has made substantial contributions to the conception and design, or acquisition of data, or analysis and interpretation of data as well as carried out the molecular genetic studies. ARC has made substantial contributions to the conception and design, or acquisition of data, or analysis and interpretation of data, and performed clinical examination of the animal. ALAS and FHEA have made an analysis of the histopathological examination and are involved in drafting the manuscript or revising it critically for important intellectual content. ISO has made substantial contributions to the conception and design, or acquisition of data, or analysis and interpretation of data, and performed histopathological examination. RMSN has been involved in drafting the manuscript or revising it critically for important intellectual content and has given final approval of the version to be published. APC conceived of the study and participated in its design and coordination and helped to draft the manuscript, gave final approval of the version to be published and participated in the sequence alignment and drafted the manuscript.

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### **Statement of Informed Consent**

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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## ***ACANTHOCHEILONEMA RECONDITUM* (SPIRURIDA: ONCHOCERCIDAE) U USNOJ DUPLJI PSA U SEVEROISTOČNOM BRAZILU: PRIKAZ SLUČAJA**

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Jedanaestogodišnji mužjak psa sa egzoftalmijom i perforacijom rožnjače levog oka doveden je radi pregleda u veterinarsku bolnicu Državnog univerziteta Maranhao. Prilikom kliničkog pregleda na jeziku psa uočeni su granulomi nepravilnog oblika zrnastog izgleda. Pas je poslat na operaciju enukleacije oka. Sprovedene su sledeće analize: kompletna krvna slika, biohemija seruma, ispitivanje na hemoparazite, rendgenski snimak grudnog koša, ehokardiogram, elektrokardiogram, citologija i histopatologija nodula. U krvnoj slici je utvrđena neutrofilija, znaci anemije, povećanje ukupnog proteina plazme i trombocitopenija. Biohemija seruma i imidžing dijagnostika su pokazali normalne rezultate. Citološkim pregledom čvorova na jeziku utvrđene su mikrofilarije, a histopatološkom analizom hronični glositis. Na uzorku krvi izvršena je polimeraza lančana reakcija (PCR) i genetsko sekvencioniranje, što je omogućilo identifikaciju vrste *Acanthocheilonema reconditum*. Ovo je prvi zabeleženi slučaj u svetu mikrofilarije *A. reconditum* u čvorovima na jeziku. Ovaj nalaz služi da upozori dermatologe i stomatologe na potrebu za diferencijalnom dijagnozom za oblike lezija usne duplje nepoznate etiologije.