









Granuloma caused by *Acanthocheilonema* spp. in a canine – case report

[Granuloma por *Acanthocheilonema* spp. em um cão – relato de caso]

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ABSTRACT

A canine patient, 13-year-old mixed breed male, 24kg, was seen at a private clinic in the city of Guayaquil, Ecuador. The main reason for consultation was the evaluation of a mass in the right knee and neck that had previously been cytologically diagnosed of neoplastic origin by another team of veterinarians through fine needle puncture and cytology. The contacts were asymptomatic. At the time of the consultation, a detailed exploration and complementary methods were performed that included hematological and urinary exams, mass cytology, serological test, chest radiography and biopsy for further histopathological study. The final diagnosis was a cutaneous granuloma due to microfilariae of *Acanthocheilonema* spp. and its subsequent surgical and medical treatment with excellent results. The canine heartworm is a cosmopolitan disease caused by nematodes of the genus *Dirofilaria*, with *D. immitis* being the best-known species. Less frequently, the species *Acanthocheilonema* spp., found in this patient, is also reported in Americas, with little knowledge about it. This is the first report of its kind hoping that it will serve as a basis for future studies.

Keywords: dermatitis, heartworm, pruritus, vasculitis

RESUMO

Foi atendido, em uma clínica particular, na cidade de Guayaquil, Equador, um paciente canino, sem raça definida, de 13 anos de idade, macho, não castrado, de 24kg. O motivo da consulta foi a avaliação de uma massa em região de articulação de joelho direito e de pescoço, que havia sido previamente diagnosticada como de origem neoplásica por outra equipe de médicos veterinários, mediante punção por agulha fina e citologia. Os contactantes eram assintomáticos. No momento da consulta, realizaram-se detalhadas avaliações clínica e complementar, que incluíram exames hematológicos, urinálise, citologia da massa, teste sorológico, radiografia torácica e biópsia para posterior estudo histopatológico. O diagnóstico final foi granuloma cutâneo por microfilária de *Acanthocheilonema* spp., para o qual os tratamentos clínico e cirúrgico apresentaram excelentes resultados. A dirofilariose é uma enfermidade cosmopolita causada por nematódeos do gênero *Dirofilaria*, sendo a espécie *D. immitis* a mais conhecida. Em menor frequência, a espécie *Acanthocheilonema* spp. encontrada neste paciente é também relatada na América, existindo pouco conhecimento a seu respeito. Este é o primeiro relato de caso sobre este tema e espera-se que sirva como base para futuros estudos.

Palavras-chave: dermatite, dirofilaria, prurido, vasculite

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INTRODUCTION

Filarial worms have a worldwide importance in mammal health, being the dogs definitive host for numerous species, such as *Acanthocheilonema reconditum*, *Dirofilaria immitis*, *Dirofilaria repens*, *Cercopithifilaria baina*, *Cercopithifilaria grassii* and *Onchocerca lupi* (Otranto et al., 2013). The species of *Acanthocheilonema reconditum* (*A. reconditum*), *A. dracunculoides*, *Cercopithifilaria baina* and *Onchocerca lupi*, are reported in America, with little knowledge regarding their clinical potential (Dantas-Torres and Otranto, 2020).

Regarding subcutaneous canine vector-borne helminth (CVBH), *A. reconditum* and *D. repens* affect canines and cause dermatological lesions. *A. reconditum* is transmitted by fleas, lice, and ticks (Harguis et al., 1999); *A. dracunculoides* are transmitted by fly louse and ticks, and *D. repens* by mosquitoes, *D. repens* tends to be described in South America, only in Brazil and *A. reconditum* is widely distributed worldwide and in South America. *A. dracunculoides* is widely distributed in Europe, Asia, Africa (Dantas-Torres and Otranto, 2020) and very rarely reported in the Americas (López et al., 2012).

Canines are the definitive hosts for *Acanthocheilonema* spp. *A. reconditum* adult parasites are in subcutaneous tissue and fascia, while *A. dracunculoides* is an abdominal cavity parasite, the microfilariae of these are found mainly in blood (Dantas-Torres and Otranto, 2020). Invasion of the cutaneous by filarial worms can manifest itself as small subcutaneous nodules, rarely painful or itchy, within which the adults and microfilariae are localized (Ferasin and Knight, 2005). The course of infection begins when the vector borne L3 larvae develop into an adult in the subcutaneous tissue in 2 to 3 months (Lindeman et al., 1983).

Conventionally, the diagnosis of *A. reconditum* is based on the presence of adult nematodes in the subcutaneous fascia of the limbs or in the material of the subcutaneous nodules (mainly post-mortem findings) and the detection of microfilariae in peripheral blood, the diagnosis of *A. dracunculoides* is similar (Bowman, 2014). A modified Knott test can be used to detect

microfilariae in peripheral blood when subcutaneous filarial worms is suspected (Ferasin and Knight, 2005). However, circulating blood microfilariae are observed only occasionally, due to the presence of low densities in microfilariae blood (Bowman, 2014). The morphometry of the microfilariae to microscopic evaluation does not allow the differentiation between *A. reconditum* and *A. dracunculoides* because their ranges are superimposed (Magnis et al., 2013).

Nonetheless, as a differential diagnosis of various types of skin and subcutaneous lesions of unknown etiological origin, canine vector-borne helminth disease should be considered. Any mass that is found should have a cytological study performed and thus differentiate lesions of inflammatory, neoplastic, or mixed origin (Dzimira and Prządka, 2020). *Acanthocheilonema reconditum* and *A. dracunculoides* are described as a non-pathological parasite, their clinical importance is that they are confused with a *D. immitis* infection. Not much is known about the clinical findings and signs caused by these parasites in the definitive host, although pruritus can be found in patients with *A. reconditum* (Bobade and Ojebuobah, 1981). Infections with *Acanthocheilonema* spp. report dermatological manifestations such as erythema, papules, focal or multifocal alopecia (López et al., 2012). The purpose of this report is to present a case of a canine with a granuloma caused by *Acanthocheilonema* spp. through the diagnosis of cytological and histopathological tests and its subsequent medical-surgical treatment.

CASUISTRY

A 13-year-old mix breed healthy male patient presented for consultation; the reason for consultation was a second opinion about a mass in the right knee that had previously been cytologically diagnosed as a soft tissue sarcoma. The patient lives outside of the house in the yard, with four other healthy dogs. The diet is with occasional commercial and homemade food, up-to-date vaccinations, delayed deworming, a history of otitis without definitive diagnosis and flea injuries, there is also a history of an accident, a run over with injuries to the spine and hip, there are no trips abroad and currently, the

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owners also noticed an increase in water consumption and urination volume.

The patient presented a 2/5 body condition, shaggy hair, normal mental attitude, pink, moist oral mucosa, with a capillary filling time of 2 seconds, normal submandibular lymph nodes, respiratory rate 30 per minute, heart rate 130 beats per minute. Abdominal palpation with any abnormalities, presented pain when manipulating

the hindquarters due to chronic lumbo-sacral degeneration, rectal temperature was 39.5°C.

A 5 cm mass was observed in the right hind limb in the knee area (Fig. 1 B), and a 3 cm neck mass that was not reported by the owners. The masses were semisolid in consistency, broad based, slow growing, regular, and not vascularized, they were very similar anatomically.



Figure 1. Clinical presentation of Granuloma by *Acanthocheilonema* spp. in the patient. A) Image of the patient at the time of consultation. B) Image of the 5cm subcutaneous mass in the right hind limb (White arrow).

Within the differential diagnoses, cutaneous neoplasia, abscess, cysts, and hematomas were considered. The recommendation was to carry out blood and urine tests and repeat cytology studies. Microcytosis and thrombocytopenia were found in the blood count. Microcytosis alters the hematocrit (falsely lowering it) and thus also lowering hemoglobin. One of the most common causes of microcytosis is chronic inflammation, which is consistent with this clinical case. In the biochemical profile, an increase in globulins was observed; It is important to highlight that globulins have 3 different fractions, of which beta-globulins tend to increase due to suspicion of proteinuria of renal origin; the presence of ketone bodies without diabetes was due to a diet high in carbohydrates or to the patient's prolonged fasting (anorexia, hyporexia), there is aminoaciduria, which increases the suspicion of tubular proteinuria. In the urine examination,

proteins were observed, which without sediment become of diagnostic value. Urinary specific gravity was low, consistent with chronic renal tubular damage, so it's possible that the patient is considered chronic kidney disease IRIS 1.

Fine needle aspiration (FNA) cytology of the masses was performed with Diff Quick staining. At a lower magnification under the microscope, a hemorrhagic background was evidenced with microorganisms with larval characteristics suggestive of microfilariae in various numbers, a high number of degenerate neutrophils and eosinophils with an infiltrate of a small number of mesenchymal cells. In greater magnification, a morphometric evaluation of the length of the body and diameter, shape of the cephalic area and tail of the microfilariae was carried out, taking the references of Magnis *et al.* (2013), showing a diameter of the length of the body of 222µm and width 5µm, to measure a

technological tool that comes with the digital microscope was used. The cephalic area is rounded, and its tail ended in a hook shape.

Suggestive findings of *Acanthocheilonema* spp. (Fig. 2).

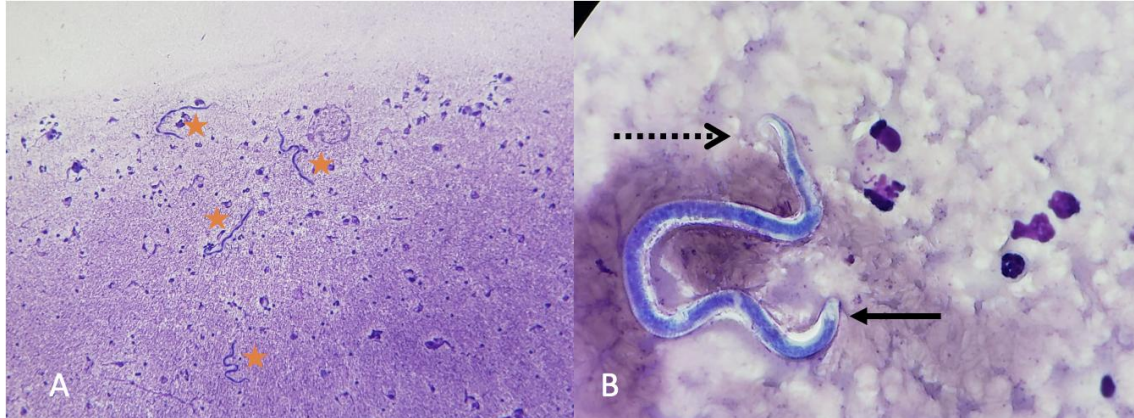


Figure 2. FNA cytology of the mass of the right hind limb A) Several microfilariae (orange star), D.Q. 10x lens. B) Image of the morphology of the microfilaria of *Acanthocheilonema* spp., The cephalic area is rounded (Arrow) and its tail ends in a small hook (discontinuous arrow). Degenerate neutrophils are observed next to it. D.Q. 100x lens.

The owner was informed of results obtained and additional diagnostic aids were recommended to subsequently perform surgery on the 2 masses for a pathological study. A 4Dx test of Idexx and Knott was performed to rule out infection by *D. immitis*, yielding a negative result for both. Finally, chest X-rays are taken, showing osteoarthritis, intervertebral disc disease and spondylosis between T6/T7 and T11/T12. Apparent fusion of the dorsal articular processes of T10/T11 and T11/T12. In both scapulohumeral joints were found sclerosis of the articular surfaces, irregular joint space, osteophyte formation in the caudal aspect of the humeral head, osteoarthritis in both scapulohumeral joints. Diffuse generalized unstructured broncointerstitial lung disease (compatible with senile process), emphysema of the accessory and cranial lobe, mild to moderate. Possibly compensatory. Mild sternal lymphadenomegaly.

After completing the complementary studies, the patient is admitted to hospitalization for surgery and the corresponding pathological study.

Macroscopic description: The two masses were prepared in 6 portions of approximately 3cm in diameter: the mass of the RHL (Right hind limb) was black in color, with an irregular surface, firm

when cut and with a black cut surface with white dots and a cavernous appearance.

The neck mass prepared in 6 portions had similar characteristics: irregular surface, white in color, firm when cut and with cut surface of the same color (Fig. 3).



Figure 3. Preparation of the mass with sections to send to the pathological study.

Microscopic description/Hematoxylin-Eosin (HE): Microscopic description of the neck mass is one of infiltrative growth that involves the dermis and subcutis, moderately cellular, well defined, encapsulated and without compromising surgical borders. The mass is formed by an infiltration of abundant lymphocytes, plasma cells, active macrophages, and multinucleated cells (peripheral nuclei) and in a smaller quantity of neutrophils and few eosinophils, located centrally in the mass and in perivascular areas (vasculitis). The inflammatory infiltration also surrounds hemorrhagic areas in which few parasitic structures are observed, compatible with microfilariae. All this is surrounded by a marked desmoplastic reaction of fibrocytic, fibroblastic and vascular tissue (capsule). The observed histopathological pattern, the presence of larval parasitic structures compatible with microfilaria, and the macroscopic characteristics and location of the mass are compatible with cutaneous microfilarial granuloma.

The microscopic description of the mass of the RHL, is an irregular mass of expansive growth, which involves the dermis, sparsely cellular, well defined, not encapsulated and with involvement of the lateral surgical edges and not in depth. Mesenchymal growth pattern in bundles, which surround large vascular cavities, and which are in turn surrounded by moderate fibrous stroma with marked perivascular mononuclear infiltration (vasculitis). Mesenchymal/endothelial neoplastic cells do not exhibit characteristics of cellular atypia neither mitotic figures were observed. The presence of numerous larval parasitic structures is observed in the endothelial lumen of several vascular caverns, the absence of characteristics of cellular atypia and of mitotic figures. The presence of numerous larval parasitic structures in the endothelial lumen of the vascular caverns and the macroscopic characteristics and location of the mass are compatible with cutaneous hemangioma/ microfilaremia (Fig. 4 and 5).

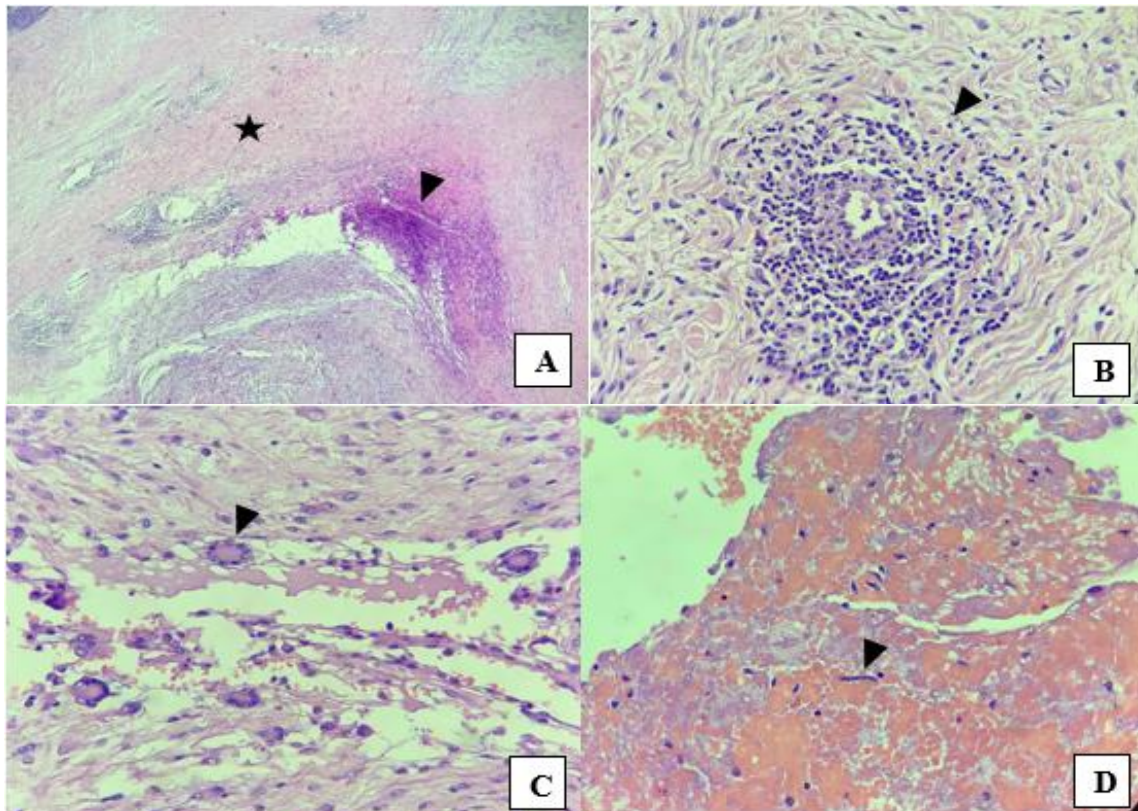


Figure 4. Histopathology of the neck mass. A) Central inflammatory infiltration (arrowhead) surrounded by abundant fibrocytic, fibroblastic and vascular tissue (star). HE 4X. B) Perivascular and vascular lymphoplasmacytic infiltration (arrowhead). HE 40X. C) Giant multinucleated cells with peripheral nuclei (arrowhead), typical of a granulomatous reaction. HE 40X. D) Larval parasitic structures compatible with microfilariae (arrowhead). HE 40X.

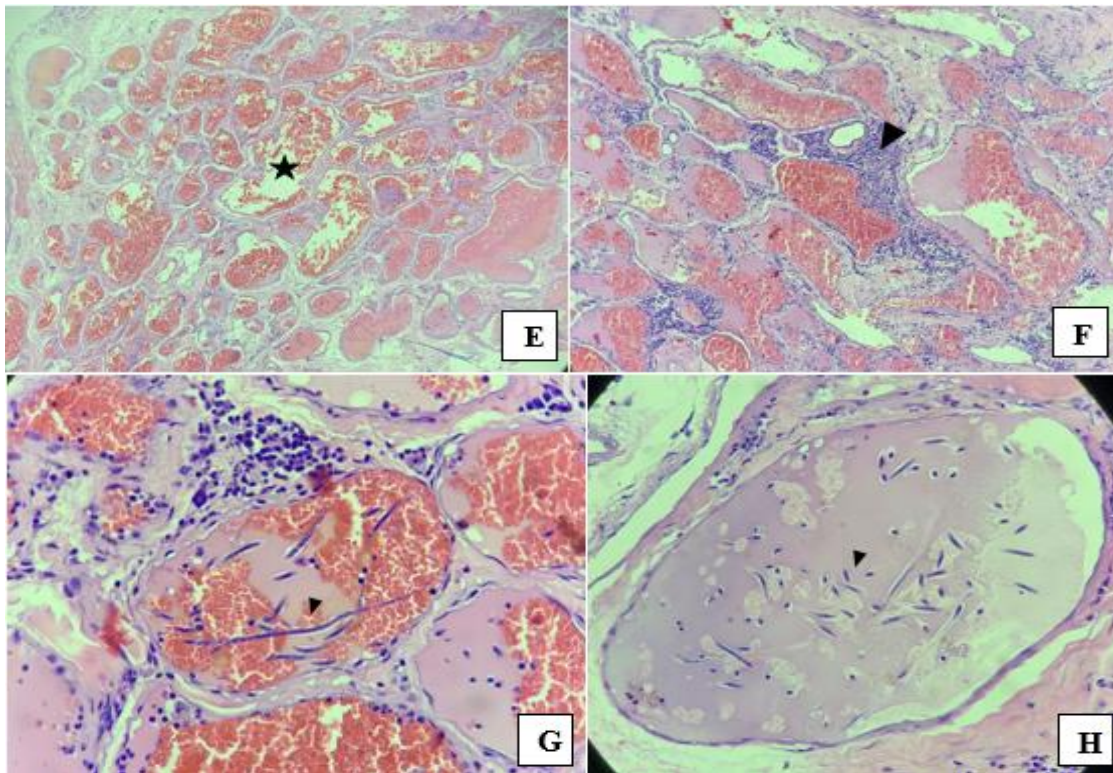


Figure 5. Histopatology of the mass of the right hind limb E) Mesenchymal growth surrounding vascular cavities (star) in lower magnification image. HE 4X. F) Marked perivascular and vascular lymphoplasmacytic infiltration (arrowhead). HE 10X. G) Larval parasitic structures (arrowhead) in the endothelial lumen of various vascular caverns. HE 40X. H) Same as image (C). HE 40X.

The medical treatment for the patient was surgery, antibiotics, and analgesics.

DISCUSSION

Heartworm caused by *Acanthocheilonema* spp. has been reported in dogs in the Americas, a lot of them are from North America and to a lesser extent, in South America, within these countries: Brazil, Chile, Costa Rica (Dantas-Torres and Otranto, 2020) and Colombia (Espinosa et al., 2020). In Ecuador there are reports on *D. immitis* only (Jimenez et al., 2020) and there are no reports as far about *Acanthocheilonema* spp.

It is important to say that currently, these parasites have been described as insignificant and they do not have clinopathological abnormalities (Dantas-Torres and Otranto, 2020). Even though *A. reconditum* is lodged during its life cycle in the skin, there are few reports of dermatological clinical signs (Harguis et al., 1999) or systemic. The dermatological clinical

sign described is the presence of pruritus (Bobade and Ojebuobah, 1981), erythema, papules, focal or multifocal alopecia (López et al., 2012), but not the formation of cutaneous granulomas as described in this clinical case.

Regarding the diagnosis of *Acanthocheilonema* spp. it is complex, since there is no method for detecting adult parasites, only by necropsy (Bowman, 2014), and the best diagnostic tool is through the microscopic study of circulating microfilariae in blood, evaluating their morphology and diameter, but the charge is low in blood and sometimes undetectable. As it is described in this case that no microfilariae were found in blood, what is surprising is the detection in skin granulomas, alterations that are reported in lesions caused by *D. repens* (McCall et al., 2008), but that are not reported in South America, except one case in Brazil (Dantas-Torres and Otranto, 2020).

The clinical significance of *Acanthocheilonema* spp. should be noted. The confusion is with the diagnosis of *D. immitis*; a parasite that affects the respiratory and cardiac system in dogs, in turn, concomitant infections have been described between *D. immitis* and *A. Reconditum* (Magui *et al.*, 2012), a disease that was ruled out in our patient, resulting negative for worm disease heart. The microfilariae can be differentiated through their morphometric evaluation (Magnis *et al.*, 2013), the microfilariae of *Acanthocheilonema* spp. are shorter than those of *D. immitis* and *D. repens*, in addition, the microfilariae of *Acanthocheilonema* spp. the cephalic end is rounded, and the tail has a hook (Magnis *et al.*, 2013). The morphometric evaluation was carried out in the FNA of the mass, not in blood since the patient was amicrofilaremic, showing that the microfilariae found in the mass were 222µm long and 5µm wide, the cephalic area is rounded and its tail ended hook-shaped, findings suggestive of *Acanthocheilonema* spp.. Differentiation between *A. reconditum* (264.8±5.5 µm x 4.6±0.5µm) and *A. dracunculoides* (259.4±6, 7µm and 5.1±0.5µm), due to their overlapping ranges, but they can be differentiated from *D. immitis* and *D. repens* (Magnis *et al.*, 2013). Although, a study in South America, described that *A. dracunculoides* is smaller (190-247µm long and 4-5µm in diameter) with respect to *A. reconditum* (260-283µm long and 5µm in diameter) (López *et al.*, 2012). In this patient, due to the lack of consensus in morphometry, it was not possible to differentiate between *A. reconditum* and *A. dracunculoides*, but according to their life cycle and that *A. reconditum* is the most frequent filaria with dermatological alterations (Hargis *et al.*, 1999), for this reason, we consider that it was the cause of the cutaneous granuloma in our patient.

Dermatitis associated with nematodes of the Onchocercidae family in dogs is rare and can be caused by *D. immitis*. Adult parasites have been found in nodular skin lesions especially on the extremities, associated with pruritic papulonodular or plaque-like dermatitis on the peripheral parts of the body, especially the head and extremities (Mozos *et al.*, 1992). The lesions found in dogs infected with *D. immitis* and *A. reconditum* are papules, plaques, with alopecia, scabs, erythema, ulceration, and scars, most of them presented itching, the lesions were located

on the neck, head, shoulder, back and belly (Harguis *et al.*, 1999), lesions not described in our patient but the presence of two cutaneous granulomas in the neck and RHL.

Histologically, dermatitis associated with microfilariae is characterized by the presence of perivascular inflammatory cells, with a variable number of eosinophils (Harguis *et al.*, 1999), in our case the masses were histologically characterized by an infiltration of abundant lymphocytes, plasma cells, active macrophages and multinucleated cells with fewer neutrophils and few eosinophils, located centrally in the mass and also in perivascular areas (vasculitis), lesions compatible with cutaneous granuloma. In turn, it has also been described that dogs associated with microfilariae dermatitis may present perivascular or periglandular inflammation with mixed inflammatory cells, causing microgranulomas, containing microfilariae or remains of them, microfilariae are associated with causing a chronic inflammatory process on the skin causing granulomatous inflammation, a possible cause of the cutaneous granulomas of this patient (Harguis *et al.*, 1999).

CONCLUSION

In conclusion, the morphometric analysis performed in the FNA of the mass turned out to be extremely useful as a quick and inexpensive diagnostic tool and represents the first step in the diagnosis of a mass (Dzimira and Przada, 2020). However, discrimination between different filarial species can be challenging, in these cases molecular methods (Espinosa *et al.*, 2020) or histochemical staining (Peribanez *et al.*, 2001) are required for discrimination. The dermatitis associated with *Acanthocheilonema* spp. may be related to type I hypersensitivity, although more studies are needed on the role these parasites play in allergic or atopic patients (Harguis *et al.*, 1999).

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