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Chronic polyarthritis associated to *Cercopithifilaria baina* infection in a dog

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Abstract

Despite the widespread distribution of *Cercopithifilaria baina* among canine and tick populations worldwide, this filarioid is currently considered of ‘minor importance’ in veterinary medicine, particularly when compared to related filarioids, such as *Dirofilaria immitis* and *Dirofilaria repens*. To date, only a single case of dermatological alterations possibly associated to infestation by *C. baina* had been reported in a dog. In the present study, we describe the first case of systemic alterations associated to *C. baina* infestation in a dog suffering from diffused chronic polyarthritis. The animal had a previous history of reluctance to movement and stiff gait and displayed multiple joint pain during manipulation of limbs. No biochemical, haematological and x-ray alterations were detected; microfilariae were observed in the synovial fluids collected from the joints. In spite of the morphological and molecular identification of these microfilariae as *C. baina*, the dog did not respond to multiple microfilaricidal treatments with milbemicyn oxime. The potential role of *C. baina* in the pathogenesis of this clinical condition is discussed. Given the potential pathogenicity of this parasite, improved knowledge of this little known tick-borne transmitted nematode is warranted in order to assist the development of novel and effective treatment strategies.

Keywords: *Cercopithifilaria baina*, synovial fluid, polyarthritis, dog

1. Introduction

Canine filarioses by skin dwelling microfilariae, such as those within the genus *Cercopithifilaria* (Spirurida, Onchocercidae) are tick-borne infestations characterised by a worldwide distribution (Otranto et al., 2013a). Dogs may be infected by at least three species of *Cercopithifilaria*, namely *Cercopithifilaria grassii*, *Cercopithifilaria bainaie* and a third species, *Cercopithifilaria* sp. II sensu Otranto et al. 2012, whose adults are yet to be described (Otranto et al., 2013b). *Cercopithifilaria bainaie* is prevalent in both canine and tick populations from the Mediterranean area (i.e., Spain, Greece and southern Italy) (Otranto et al., 2013b), as well as Australia, Brazil, Malaysia and South Africa (Almeida and Vicente, 1984; Latrofa et al., 2014).

The distribution of canine *Cercopithifilaria* spp. is strictly associated with that of their main arthropod vector, i.e. *Rhipicephalus sanguineus* sensu lato ticks (Brianti et al., 2012; Ramos et al., 2014a). In spite of its broad geographical distribution, *C. bainaie* has traditionally been considered of minor importance to canine health, particularly when compared to the related species *Dirofilaria immitis* and *Dirofilaria repens*, the causative agents of heartworm and subcutaneous filariosis, respectively (Otranto et al., 2013b). In fact, besides evidence that *C. bainaie* infestation can occur together with infections by other tick-borne microorganisms (i.e., *Anaplasma platys*, *Babesia vogeli* and *Hepatozoon canis*) (Ramos et al., 2014b), the pathogenicity of *C. bainaie* remains largely unknown. In one single report, a dog infested by *C. bainaie* was presented with erythematous, papular and pruritic dermatitis (Otranto et al., 2012). Upon histological examination of the skin, congestion of the superficial plexus and mild focal epidermal/subepidermal oedematous changes were observed, in association to perivascular and interstitial dermatitis and inflammatory infiltrates (i.e., neutrophils, eosinophils and lymphocytes), surrounding the microfilariae (Otranto et al., 2012).

In the present study, we describe the first case of possible systemic alterations caused by *C. bainaie* in a dog suffering for diffused chronic polyarthritis. Microfilariae of *C. bainaie* were

detected at the cytological examination of the synovial liquid and its potential role in the pathogenesis of this clinical condition is discussed.

2. Materials and methods

In July 2013, a 7-year old mixed breed dog living in the municipality of Viterbo (Lazio region, central Italy) was admitted to a private practice with a history of reluctance to movement, lethargy and lameness. The animal had spent two months (May-June 2013) in the Tuscany region with its owner, who reported a history of tick infestation. Chewable tablets containing ivermectin/pyrantel (Cardotek plus, Merial, France) had been previously administered for the prevention of cardiopulmonary filariasis. At the clinical examination, the dog displayed stiff gait and abnormal posture, as well as pain of multiple joints during the manipulation of both fore and hind limbs.

Biochemical and haematological parameters were within the normal species range and the animal tested negative for *Leishmania infantum* infection using a rapid kit (SNAP[®] Leishmania Test, IDEXX Laboratories, USA). Based on this clinical presentation, a diagnosis of chronic polyarthritis was made and two cycles of anti-inflammatory symptomatic treatment were administered once daily for a week in July (i.e., 0.1 mg/kg meloxicam, Metacam, Boehringer Ingelheim, Germany) and August (1 mg/kg robenacoxib, Onsior, Novartis, USA), respectively. In September, following the deterioration of its clinical status, the animal was treated with corticosteroids (1 mg/kg prednisone, Vetsolone, Bayer, Italy), daily for 10 days, resulting in a temporarily partial recovery.

In January 2014, x-rays of the main joints were performed, in order to investigate the origin of the painful stimulus during the limb manipulation; however, no articular, muscular and bone alterations were observed. Therefore, synovial fluid from the shoulder, elbow, hip, knee and tarsal joints was collected by fine-needle aspiration, and parameters of inflammation were assessed (first sampling) (Table 1). Upon microscopical examination of the articular fluids, an increased presence of mononuclear cells was observed, with >10% appearing degenerated. In addition, live and active

moving microfilariae, resembling those of *C. bairdii* (Otranto et al., 2013c), were also detected. To confirm the morphological identification, genomic DNA was extracted from the skin sample, as well as from single microfilariae collected from the synovial fluid, using a commercial kit (ArchivePure DNA Tissue Kit, 5 Prime, Gaithersburg, USA).

All samples were molecularly processed for specific amplification of the partial cytochrome oxidase subunit 1 (*cox1*) gene fragment (~304 bp) targeting *Cercopithifilaria*, using specific primers (CbCox1F/COIntR), reaction procedures and an amplification protocol previously described (Otranto et al., 2011; Otranto et al., 2013c). All amplicons were purified using Ultrafree-DA columns (Amicon, Millipore; Bedford, USA) and sequenced directly using the Taq DyeDeoxyTerminator Cycle Sequencing Kit (v2, Applied Biosystems) in an automated sequencer (ABI-PRISM 377). Sequences were aligned using the ClustalW program (Larkin et al., 2007), and compared with those available in GenBank™ dataset by Basic Local Alignment Search Tool analysis (Altschul et al., 1997). In the meantime, during a x-ray follow-up, an intra-thoracic mass was observed in the mediastinum and in the right pulmonary lobe. A biopsy was collected and a diagnosis of thymoma was made.

In February 2014, the dog was specifically treated against microfilariae with milbemycin oxime (Milbemax, Novartis, Switzerland), administered orally at the dose of 0.5 mg/kg, once every 7 days for 3 weeks. One month later, during the surgical removal of the neoplastic tumour, synovial fluids from the same joints were collected (second sampling), in order to evaluate the efficacy of the microfilaricidal treatment. A skin sample was also collected from the inter-scapular area, soaked in saline solution for 10 min at 37°C. A few drops of the sediment (i.e., 40 µl) were observed under the light microscope (100x magnification) after the addition of a drop of methylene blue (1%) (Otranto et al., 2011). The dog died one day after the surgery due to post-surgery complications.

3. Results

Microfilariae, detected in the articular fluids, were identified as *C. bairnei* (Fig. 1) based on measurements (i.e., $182.5 \pm 2.9 \mu\text{m}$ in length and $8 \pm 1.2 \mu\text{m}$ in width) and morphological features (i.e., rounded head, short dorso-ventrally flattened body, thick cuticle featured by conspicuous transverse striations). PCR of the skin sample (only) yielded amplicons which, when sequenced, matched *C. bairnei* 'haplotype I' (GenBank AN: JF461457). At the second sampling, microfilariae of *C. bairnei* were detected in synovial fluids collected from the right joints (Table 1).

4. Discussion

The clinical case described herein provides support to the hypothesis that *C. bairnei* may cause polyarthritis in dogs. Other causes of polyarthritis, including infection by *Borrelia burgdorferi* in which cytopathological findings are typically characterized by a cell count of $\sim 12,700$ cells/microliter (with $\sim 97\%$ neutrophils) (Valenciano and Cowell, 2013) were excluded; indeed, the synovial fluid collected from the dog examined was characterized by an increase in mononuclear cell number, including $>10\%$ degenerated cells.

This neglected filarioid species has recently attracted the interest of the scientific community, due to its widespread distribution amongst canine populations worldwide (Otranto et al., 2011; Ramos et al., 2013, 2014a, 2014b; Latrofa et al., 2014; Solinas et al., 2014). However, thus far, limited information is available on the pathogenicity of this parasite. Indeed, the 28 *Cercopithifilaria* species that parasitize mammals (i.e., ruminants, cercopithecoid primates, carnivores, rodents and marsupials) are known to cause relatively benign infestations, with no major clinical alterations usually observed in infested animals. However, some generalized lesions (e.g., in the subcutaneous, vascular and ocular regions) have been described in the captive-bred bush rats (*Rattus fuscipes*) and short-nosed bandicoots (*Isodon macrourus*) infected by *Cercopithifilaria johnstoni*, as well as in the African porcupine (*Hystrix cristata*) parasitized by *Cercopithifilaria roussilhoni* (Voung et al., 1985; Voung et al., 1993).

The finding of *C. bairnei* microfilariae in the synovial liquid of a dog with polyarthritis could lean for its potential pathogenic role in the pathogenesis of this articular disease. While it has already been suggested that microfilariae may disseminate *via* the lymphatic vessels (Bain et al., 1994), the causative role of *C. bairnei* in joint disease remains to be addressed (e.g., by a careful appreciation of the joint histological lesions). The localization of adult *C. bairnei* and the distribution of microfilariae in the host body has been recently assessed and demonstrated to mainly involve the subcutaneous areas of the trunk and inter-scapular region, respectively (Otranto et al., 2012; Otranto et al., 2013c). Therefore, the presence of *C. bairnei* in the synovial fluid may be considered an aberrant localization caused by an underlying inflammatory condition of the joint tissues. However, the persistence of multiple joint pain, also following the microfilaricidal treatment with milbemycin oxime, at dosage and treatment protocol effective for filarioids of the genus *Dirofilaria* (Tagawa et al., 1993; Rawlings et al., 2001; Nolan et al., 2012), raises questions on the efficacy of this treatment against synovial infection. The detection of inflammatory and degenerated cells in the articular fluid could also be associated to the chemotaxis of neutrophils stimulated by *C. bairnei* at the dermis level (Otranto et al., 2012). *Cercopithifilaria bairnei* could have also caused a local lymphangitis, due to a pathogenic mechanism similar to the inflammatory reactions and acute vasculo-exudative response with fibrosis induced by *C. johnstoni* in the perivascular connective tissues of infected animals (Vuong et al., 1993).

Based on the anamnesis, the dog had most likely been infected by *C. bairnei* during May-June 2013. Indeed, the pre-patent period of microfilariae occurrence fits with previous evidence of infection in young dogs, i.e. as early as 3 months following tick infestation (Ramos et al., 2014b). Finally, the failure of the systemic treatment against microfilariae of *C. bairnei* must also be noted. Further studies are warranted in order to elucidate the actual pathogenic role of this canine filarioid, as well as effective treatment and control strategies against this little known tick-borne nematode of dogs.

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167 **Competing interests**

168 The authors declare that they have no competing interests.

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237 **Legend to figure**

238 **Figure 1.** Microfilaria of *Cercopithifilaria binae* detected in the synovial fluid following the right

239 shoulder joint centesis (scale bar: 50µm).

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Table 1. Number of *Cercopithifilaria baina* microfilariae found in 40 µl of articular fluid at the first and second sampling.

Joint	First sampling	Second sampling
Right shoulder	1	8
Right elbow	5	2
Right carpus	-	1
Right tarsus	-	1

Figure 1