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Short Communication

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

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ABSTRACT

Despite the widespread distribution of *Cercopithifilaria bainae* 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 infection by *C. bainae* had been reported in a dog. In the present study, we describe the first case of systemic alterations associated to *C. bainae* infection in a dog suffering from diffused chronic polyarthritis. The animal had a previous history of reluctance to move 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. bainae*, the dog did not respond to multiple microfilaricidal treatments with milbemicyn oxyme. The potential role of *C. bainae* in the pathogenesis of this clinical condition is discussed. Given the potential pathogenicity of this parasite, improved knowledge of this little known tick-borne nematode is warranted in order to assist the development of novel and effective treatment strategies.

1. Introduction

Canine filarioses by skin dwelling microfilariae, such as those within the genus *Cercopithifilaria* (Spirurida, Onchocercidae) are tick-borne infections characterized by a worldwide distribution (Otranto et al., 2013a). Dogs may become infected with least three species of *Cercopithifilaria*, namely *Cercopithifilaria grassii*, *Cercopithifilaria bainae* and a third species, *Cercopithifilaria* sp. II sensu Otranto et al. (2012), whose adults are yet to be described (Otranto et al., 2013b). *C. bainae* is prevalent in both canine and tick populations from the Mediterranean area (i.e., Spain, Greece and

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southern Italy) (Otranto et al., 2013b), as well as Australia, Brazil, Malaysia and South Africa (Almeida and Vicente, 1984; Otranto 2014, unpublished data).

The distribution of canine *Cercopithifilaria* spp. is strictly associated with that of their main arthropod vectors, *Rhipicephalus sanguineus* sensu lato ticks (Brianti et al., 2012; Ramos et al., 2014a). In spite of its broad geographical distribution, *C. bainae* 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. bainae* infection 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. bainae* remains largely unknown. In one single report, a dog infected by *C. bainae* 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. bainae* in a dog suffering from diffused chronic polyarthritis. Microfilariae of *C. bainae* were detected during 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 move, 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 as a preventative measure against cardiopulmonary filariosis. 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 temporary partial recovery.

Table 1
Number of *Cercopithifilaria bainae* microfilariae detected 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

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. bainae* (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 (*cox*1) gene fragment (~649 bp) targeting *Cercopithifilaria*, using specific primers (CbCox1F/COIintR COIF/COIR), reaction procedures and an amplification protocol previously described (Otranto et al., 2011, 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 (100× magnification) after the addition of a drop of methylene blue (1%) (Otranto et al., 2011). The dog died one day later due to post-surgery complications.