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SHORT COMMUNICATION

Cercopithifilaria sp. II in *Vulpes vulpes*: new host affiliation for an enigmatic canine filarioid

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Abstract *Cercopithifilaria bainae* and *Cercopithifilaria grassii* (Spirurida, Onchocercidae) are filarioids inhabiting the skin of dogs worldwide. The microfilariae of a third species namely, *Cercopithifilaria* sp. II sensu Otranto et al. 2013, have been morphologically and molecularly characterized but scientific knowledge of this parasite is minimal. The first case of infection of a red fox (*Vulpes vulpes*) with the filarioid *Cercopithifilaria* sp. II is herein described in Castro Marim, Portugal. Microfilariae from skin sediment of the fox's ear were morphological characterized, and the identification was confirmed molecularly in samples from skin sediment, skin samples, and from *Rhipicephalus sanguineus* group ticks collected from the animal (99% homology with *Cercopithifilaria* sp. II). Studies should evaluate if red foxes might play a role in the maintenance and distribution of *Cercopithifilaria* sp. II infection in dog populations.

Keywords *Cercopithifilaria* sp. II · *Vulpes vulpes* · Portugal

Manuscript

The genus *Cercopithifilaria* (Spirurida: Onchocercidae) comprises several tick-borne filarial nematodes parasitizing a broad range of wild and domestic mammals. Adult worms are usually localized beneath the cutaneous tissues, where they are not easily detected (adult are extremely thin and not easy to be recovered from the connective matrix) and their microfilariae are always in the dermis (Bain et al. 2002). *Cercopithifilaria bainae, Cercopithifilaria grassii* and *Cercopithifilaria* sp. II sensu Otranto et al. 2013, transmitted by *Rhipicephalus sanguineus* group ticks (Pampiglione et al. 1983; Brianti et al. 2012), have been morphologically and/or molecularly differentiated in canine populations in Europe (Otranto et al. 2012c; 2013a; Ionică et al. 2014; Solinas et al. 2014), including in the south of Portugal (Cortes et al. 2014) as well as from ticks infesting dogs (Otranto et al. 2012b; Ramos et al. 2013; Latrofa et al. 2014; Maia et al. 2014; Solinas et al. 2014). Microfilariae of these three species can be discriminated from each other by their length and general morphological features and also by molecular analysis of mitochondrial target genes (Otranto et al. 2013a).

Although this filarioid is regarded as minimally pathogenic, as most of the dogs infected with *Cercopithifilaria* spp. do not present physical abnormalities that could be associated to the presence of microfilariae in the skin, dermatological alterations (Otranto et al. 2012a) and diffused chronic polyarthritis (Gabrielli et al. 2014) without any other apparent cause, have been described in two dogs infected with *C. bainae* while gross skin lesions reported in one dog infected with *Cercopithifilaria* sp. II (Otranto et al. 2013a).

In April 2016, an adult female red fox (*Vulpes vulpes*) recovered from the municipality of Castro Marim (south of Portugal) was admitted for examination at the RIAS-Wildlife Rehabilitation and Research Centre, Olhão (Portugal). The

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animal was cachectic, with pale mucosae, ocular and vaginal purulent discharge, and highly infested by ticks and fleas. After natural dead, necropsy revealed the presence of hydrothorax, hydropericardium, splenomegaly and generalized lymphadenomegaly. At the necropsy, a skin sample of the left ear was obtained and soaked at room temperature in 1 ml of saline solution.

Sediment (20 μl) was placed on a glass slide and observed under light microscopy (magnifications of x10 and x40). Microfilariae were counted, identified according to morphologic keys (Otranto et al. 2013a), and differentiated from those of filarioid species most commonly retrieved in dogs from the Mediterranean region (Otranto et al. 2011). Briefly, microfilariae of *Cercopithifilaria* sp. II had a body length of 273-305 μm with a well-developed lateral alae, a thin anterior extremity and a short attenuated tail (Fig. 1). After microscopic observations, skin sample and sediments, as well as *R. sanguineus* group ticks (two females and two males) collected from the fox, were stored in 70% ethanol and species identification was assessed by PCR amplification and DNA sequencing. Genomic DNA was extracted from the skin sample and from microfilariae isolated from sediments using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen, Germany) in accordance with the manufacturer's instructions whereas DNA from *R. sanguineus* adult ticks was extracted as previously described (Otranto et al. 2012c). All samples were molecularly processed for specific amplification of a partial (~689 bp) cytochrome *c* oxidase subunit 1 (*cox*1) gene fragment targeting *Cercopithifilaria* spp., with NTF/NTR primers, following reaction procedures and amplification protocol described elsewhere (Otranto et al. 2011). Amplicons obtained from the sediments, skin sample and from two ticks (one male and one female) were purified using Ultrafree-DA columns (Amicon, Millipore, USA) and sequenced directly with the Taq DyeDeoxyTerminator Cycle Sequencing Kit (v.2, Applied Biosystems, USA) in an automated sequencer (ABI-PRISM 377, Applied Biosystems). The sequences were determined from both strands, using the same primers as for PCR, aligned using ClustalW program and compared among them and with those available in GenBank dataset by Basic Local Alignment Search Tool (BLAST - http://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST analysis revealed 99% homology of the amplified sequences with the closest accession number of *Cercopithifilaria* sp. II (JQ837809) available in GenBank database. Obtained sequences were deposited in GenBank under accession numbers. KX898456-KX898457.

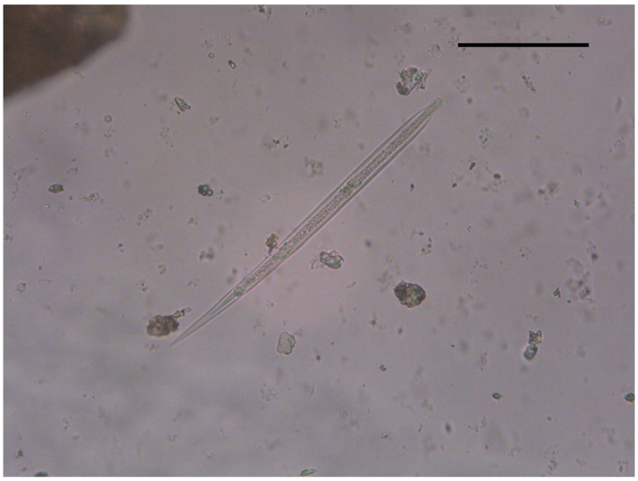

Fig. 1 Microfilaria of *Cercopithifilaria* sp. II detected in the sediment of a skin biopsy from the left ear of a red fox (*scale bar* = 100 μm)

To the authors' knowledge, this report describes the first case of infection with any *Cercopithifilaria* in a red fox. Thought this finding should not be completely surprising as it is known that foxes and dogs share many tick-borne pathogens (Otranto et al. 2015), filarioids within the genus *Cercopithifilaria* do not cross-infect different animal species being usually highly affiliated to unique hosts. However, *Cercopithifilaria* spp. are circulating in dogs and ticks from the same region (Cortes et al. 2014; Maia et al. 2014), therefore enhancing the possibility of cross infections among foxes and dogs, often infested by the same tick species.

As previously reported in most of the infected dogs (Otranto et al. 2013b; Cortes et al. 2014), the fox did not show apparent dermatological manifestations. For their habits to approach urban and rural settlements in search for food (Tolhurst et al. 2011), moving within an area ranging between 10 and 30 km^2 (Coman et al. 1991), foxes could play a role in the dissemination of *Cercopithifilaria* sp. II by acting as sylvatic carriers or reservoir hosts of this parasite. In fact, a concern about the transmission of parasitic diseases from domestic to wildlife carnivores and vice versa is increasing, especially in areas where ecological factors linked to human activities (e.g. encroachment and tourism) have contributed to a regularly interaction between them. However, as no studies have yet been conducted on the simultaneous occurrence of *Cercopithifilaria* sp. II infections in domestic and wild mammals in a given geographical area, it is not possible to define the potential interactions between domestic and sylvatic cycles. Therefore, further investigation such as population-based surveys should be performed not only to estimate the distribution of *Cercopithifilaria* sp. II infection in foxes and to assess the risk to dogs but also to check in foxes for adult nematodes in order to retrieve the adult specimens and formally describe this new species of *Cercopithifilaria*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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