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Short Communication

Filarial infections in domestic dogs in Lusaka, Zambia

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ABSTRACT

Filariae are common parasites of dogs in many parts of the world, but little is known about the status of these infections in sub-Saharan Africa. A study was carried out to determine the occurrence and species of filariae among 272 dogs in Lusaka, Zambia. Giemsa stained blood smear and Knott's concentration methods revealed microfilariae in 16 (5.9%) of the dogs. PCR confirmed that most of these dogs had *Acanthocheilonema reconditum* infection. Ten (4.0%) of the examined dogs were positive for *Dirofilaria immitis* circulating antigen (by DiroCHEK® test), but *D. immitis* microfilariae were not identified in any of the dogs and the status of this infection remains unclear. Further studies are needed to explore the occurrence of filariae in Zambian dogs and the zoonotic potential for humans.

1. Introduction

Filariae are vector-borne parasitic nematodes of which several species are of major public health importance, especially in warm climate countries (Simonsen et al., 2014). Dogs are also commonly infected with filarial parasites. Thus, infections in dogs with the mosquito-transmitted filariae *Dirofilaria immitis* and *Dirofilaria repens* are well known due to severe clinical manifestations elicited in the dogs, but also since some of the vectors are anthropophilic and may cause zoonotic transmission of the infections to humans (Genchi et al., 2007; McCall et al., 2008; Simon et al., 2009). *Acanthocheilonema reconditum*, *Acanthocheilonema dracunculoides*, *Cercopithifilaria grassi* and *Onchocerca lupi* are other widespread but less known filarial species of dogs (ESCCAP, 2012; Otranto et al., 2013a,b). Knowledge on the distribution, biology and veterinary and medical significance of these is limited, probably due to the less distinct clinical picture seen during infection and to a general lack of diagnostic expertise.

The traditional method for diagnosing filarial parasites is by finding and identifying their larvae (microfilariae) in blood or skin samples, usually after application of various staining and concentration techniques (Genchi et al., 2007; McCall et al., 2008). *D. immitis* diagnosis in dogs has been simplified in recent years following development of serological tests for detection of specific circulating antigens, and several brands of these tests are now commercially available (Klotins et al., 2000; McCall et al., 2008). Of late, molecular PCR and DNA sequencing techniques applied on blood samples have moreover proved valuable as highly sensitive and specific tools for detecting and identifying filarial infections in dogs (Casiraghi et al., 2006; Rishniw et al., 2006; Latrofa et al., 2012).

Information on the occurrence of canine filariosis in dogs in sub-Saharan Africa is scarce, and mainly stems from case reports. From a review of available literature by Schwan (2009) it appeared that all the above mentioned species have been reported from various places on the continent. A large survey for filariae in dogs in northern Kenya

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has later been documented (Albrectova et al., 2011). However, in general knowledge on the extent and distribution is limited. In Zambia, little attention has so far been given to the parasites of dogs, and no information is available on the occurrence of dog filarial infections in the country. As a starting point, the present study therefore aimed at determining the occurrence and species of filarial infections in dogs in Lusaka, Zambia.

2. Materials and methods

2.1. Study area, study design and sampling

The study was conducted in Lusaka, the capital of Zambia. The city is located in the central part of the country, at an altitude of about 1300 meters and with a human population of about 1.75 million. Lusaka experiences three main seasons: a cold and dry season from May to August (temperature range 6–25 °C), a hot and dry season from September to October (17–35 °C) and a wet season from November to April (14–30 °C). The study was conducted from April to December, 2013. Dogs aged six months and above according to owners information were routinely sampled when presented for medical consultation at the veterinary clinic at the University of Zambia School of Veterinary Medicine ($n = 125$) or other nearby veterinary clinics ($n = 76$) and during an anti-rabies vaccination campaign in one of the townships of Lusaka (Mtendere compound; $n = 71$).

The dogs were brought by their owners who were asked for permission to collect samples after explaining the purpose of the study. Dogs that were very ill or presented with parvovirus enteritis were excluded from the study. Serum and EDTA stabilized whole blood samples were collected from the cephalic vein of each dog. A structured questionnaire was used to obtain information about the dogs, including age, sex, breed, main function and main diet. Dogs were moreover examined systematically for ticks and fleas by visual inspection of the entire body (including ears and inguinal area) and palpation (to detect ticks that were not immediately visible). The body condition was recorded by using the Nestle Purina score system (Laflamme, 1997).

2.2. Examination of blood for microfilariae

Duplicate thick blood smears were prepared on glass slides from whole blood. Smears were air dried overnight, thereafter dehaemoglobinised (by soaking in tap water in vertical position for 1–2 min) and allowed to dry before being fixed with methanol for 30–60 s. Subsequently, the smears were stained for 30 min with a 1:14 Giemsa stock solution in distilled water, gently rinsed in a flow of tap water and allowed to dry. A compound microscope was used to identify and measure the microfilariae at 100–400× magnification.

Blood was furthermore examined for microfilariae with Knott's concentration technique (Melrose et al., 2000; Genchi et al., 2007). One ml of whole blood was mixed with 9 ml of 2% formalin in a conical centrifuge tube and then centrifuged for 5 min at 692 × *g*. The supernatant was removed by inverting the tube. The deposit was mixed with one drop of 1% methylene blue stain. Ten μl of the sample was transferred to a glass slide and examined under a compound microscope at 100× to identify the microfilariae. This was repeated until the entire sample in the tube had been examined.

2.3. Test for circulating D. immitis antigen

The DiroCHEK® Canine Heartworm Antigen test kit (Synbiotics Corporation, San Diego, USA), which is an enzyme linked immunosorbent assay, was used to detect circulating *D. immitis* antigen in the dog sera according to manufacturer's instructions. Briefly, 50 μl of each test sera (plus positive and negative controls) were individually dispensed into the antibody-precoated microtitre wells, and one drop of conjugate (Reagent 1) was added. After 10 minutes of incubation the wells were emptied and washed 5 times with distilled water. Two drops of substrate (Reagent 2) were then added to each well. After 5 min of incubation the results were read as positive (distinctly blue) or negative (completely clear) against a white background.

2.4. Identification by sequencing

Fourteen of the 16 blood samples identified positive for microfilariae by microscopy were further analyzed by PCR for speciation of the microfilariae. The MasterPure™ DNA Purifications Kit (Epicentre Biotechnologies) was used to extract DNA from 190 μl of the whole blood samples according to the manufacturer's protocol except that samples were incubated with 200 mg proteinase K overnight at 56 °C.

The ITS2 region was PCR amplified in a total volume of 20 μl using the primers DIDR-F1 and DIDR-R1 (Rishniw et al., 2006) under standard PCR conditions using 1 μl DNA as template and an annealing temperature of 58 °C. Likewise, a part of the mitochondrial *cox*1 gene was PCR amplified by primers COIintF and COIintR (Casiraghi et al., 2006) using annealing temperature of 48 °C. PCR products were stained using GelRed™ (Biotium) and visualized under UV light in 1.5% agarose gels. PCR products were cleaned up and sequenced in both directions using the PCR primers by Macrogen Inc. (Seoul, South Korea).

The sequences were manually checked using vector NTI (Lu and Moriyama, 2004) and aligned and trimmed in BioEdit (Hall, 1999). Sequences were compared to already published sequences in GenBank (BLAST search). For the *cox*1 sequences the genetic relationship between the unknown dog filariae were compared to *A. reconditum* (JF461456), *D. immitis* (FN391553), *D. repens* (JF461458) and *Cercopithifilaria sp.* (JF461457) using the Neighbour-Joining clustering method and the Kimura-2-parameter for distance estimation as implemented in MEGA 6.1 (Tamura et al., 2013). The stability of the topology was assessed with 1000 bootstraps. Unfortunately no *cox*1 sequences were available in GenBank for *A. dracunculoides*.

2.5. Data analysis

Parasitology, serology and dog data were analyzed using STATA version 13 (StataCorp, Texas, USA). Proportions