CASE REPORT

Ocular dirofilariosis by *Dirofilaria immitis* in a dog: first case report from Europe

A five-year-old, entire female mixed-breed dog was presented with corneal oedema and episcleral hyperaemia in the left eye. The ophthalmological examination revealed the presence of a free-swimming nematode in the anterior chamber. Circulating microfilariae were not observed by a modified Knott test nor were adult antigens detected in serum by a commercial ELISA. The parasite was surgically removed from the dog’s eye, but its anterior end was damaged during the surgery. Based on the morphology of the posterior end, the nematode was preliminarily identified as a male *Dirofilaria immitis*. The species identification was confirmed by PCR amplification and sequencing of the mitochondrial cox1 and 12S rDNA genes, using a DNA barcoding approach. Although other cases of ocular dirofilariosis by *D. immitis* have been previously recorded in Australia and the United States, the case reported herein is the first in a dog from Europe.

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**INTRODUCTION**

Canine vectorborne diseases are widespread around the world and represent a diagnostic challenge because of their wide range of clinical signs (Otranto and others 2009b). Some vectorborne pathogens (for example, *Dirofilaria immitis*, *Thelazia callipaeda* and *Leishmania infantum*) can cause a variety of ocular manifestations in dogs (Komnenou and Koutinas 2007). Ocular dirofilariosis caused by *D. immitis* has been reported in highly endemic areas such as Australia (Lavers and others 1969, Spratt and others 1971, Thornton 1978) and the United States (Eberhard and others 1977, Gutberchock and others 1981, Metcalf and others 1982, Miller and Cooper 1987, Carastro and others 1992). *Dirofilaria immitis* can cause a severe cardio-pulmonary disease in dogs and cats and pulmonary dirofilariosis is also a rare zoonosis (McCall and others 2008). *Dirofilaria immitis* is endemic in southern Europe and a number of reports have shown its recent spread to northern and north-eastern countries (Genchi and others 2009). In Italy, *D. immitis* is historically endemic in northern regions of the country (Genchi and others 2005), with a prevalence ranging from 22 to 80 per cent in dogs not treated with preventive drugs (Genchi and others 2001). However, new foci of *D. immitis* have recently been detected in southern Italy (Apulia and Calabria regions), showing the spread of this pathogen in this country (Otranto and others 2009a). This case reports an unusual case of ocular dirofilariosis in a dog from southern Italy.
acepromazine (2 mg/kg, IM), the eye was clipped and prepared for surgery. General anaesthesia was induced with propofol (5 mg/kg, IV) and maintained with isoflurane in oxygen. The cornea was incised with a crescent Beaver corneal knife and the anterior chamber of the eye flushed with sterile saline solution to float the nematode. A nematode (Fig 2) was retrieved from the dog’s eye, but the anterior end of the parasite was damaged during its removal. The dog recovered without complications after the surgery.

The remaining part of the parasite measured 22.9 mm in length and had a maximum diameter of 0.23 mm. The size (long spicule 0.35 mm and short spicule 0.19 mm) and the morphology of spicules and the number of caudal papillae (that is, four pairs of pre-anal papillae and one pair of post-anal papillae) were suggestive of a male *D. immitis*, as reported by Orihel (1961).

To confirm the species, a DNA barcoding approach was used. A piece (~2 mm) of the parasite was used for DNA extraction and then the *coxI* and 12S rDNA genes were amplified and sequenced as described elsewhere (Casiraghi and others 2004, 2006). The sequences obtained have been deposited in the EMBL Nucleotide Sequence Database (accession numbers FN391553 and FN391554). The sequence analysis showed a homology of 100 per cent with *D. immitis coxI* and 12S rDNA gene sequences deposited in GenBank (accession numbers EU169124 and EU182327).

**DISCUSSION**

Surgical removal is the treatment of choice for intraocular dirofilariosis in dogs (Komnenou and Koutinas 2007), the prognosis depending on the severity of ocular damage and on the successful removal of the nematode (Carastro and others 1992).

This case was quite unusual as the dog was born and lived in southern Italy where *D. immitis* has only recently been found (Otranto and others 2009a). Although ocular examination was suggestive of ocular
dirofilariosis, the absence of circulating microfilariae and the negative result of ELISA for heartworm antigens made the correct aetiopathological diagnosis difficult. However, it is known that in *D. immitis* infected animals antigen tests can result false-negative in the presence of low heartworm burdens or of a single male worm (Berdoulay and others 2004).

This case report confirms the usefulness of an integration of morphological and molecular tools to ensure the identification of parasites, especially of damaged specimens. It is also important to note that the risk finding parasites in ectopic locations is not restricted to highly endemic areas. Thus, veterinarians working in areas where *D. immitis* is spreading should include dirofilariosis in the differential diagnosis of ocular diseases in dogs, even if they are not microfilaraemic, or seroreactive.

References
Orihel, T. C. (1961) Morphology of the larval stages of *Dirofilaria immitis* in the dog. *Journal of Parasitology* 47, 251–262