CASE REPORT

Thelazia callipaeda ocular infection in two dogs in Belgium

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Worms were retrieved from the left eyes of two dogs presented for unilateral ocular discharge in Belgium. Morphological and molecular identification were performed and the parasites were identified as *Thelazia callipaeda*. The history suggested that the infection had been acquired in south-western France and southern Italy where the disease has been observed regularly for the last 6 and 12 years, respectively. In these two regions, the disease is considered endemic and spreading. To the authors' knowledge, this is the first case report of canine thelaziosis in Belgium.

Journal of Small Animal Practice (2012)
DOI: 10.1111/jsap.12003

Accepted: 2 November 2012

INTRODUCTION

Thelaziosis is caused by a parasitic nematode belonging to the genus *Thelazia* (Spirurida, Thelaziidae). The viviparous adult worm and larvae are found in the conjunctival fornices and nasolacrimal duct, and feed on lacrimal secretions. First-stage larvae *Thelazia callipaeda* are ingested by the fruit fly *Phortica variegata* (Diptera: Drosophilidae), which is the intermediate host found in Europe (Otranto and others 2006b). Larval development occurs in the ovarian follicles of the fly during summer. Late-stage larvae migrate to the mouthparts of the fly and are transferred to the final host when the fly feeds (Taylor and others 2007).

Thelazia callipaeda has been described in cattle, horses, cats, dogs, red foxes, wolves, European rabbits and humans (Hong and others 1995, Otranto and Traversa 2004, Otranto and others 2007, Otranto and Dutto 2008). In dogs, *Thelazia californiensis* in North America and *T. callipaeda* in Asia have been identified (Taylor and others 2007). The latter was first described in Europe, in Piedmont (Italy) 23 years ago (Rossi and Bertaglia 1989). This "oriental eyeworm" has now been detected in France (Ruytoor and others 2010), Switzerland (Malacrida and others 2008), Germany (Magnis and others 2010), Spain (Miro and others 2011), The Netherlands and Belgium (Otranto and others 2005, Janssens and Claerebout 2006).

HISTORY

A seven-year-old, spayed female golden retriever was presented to the University of Liège with a 5-month history of intermittent discharge from the left eye (OS). Two days before presentation, the owner noticed a redness with a purulent discharge OS. This dog had been living in the Lot department in the southwest of France for several years before moving to Belgium. The second patient, an 11-year-old entire male Labrador retriever, was presented with a 3-month history of discharge OS. In the past 3 years, this dog had travelled annually to southern Italy (Basilicata), and once to the northeast of France (Alsace).

Clinical examination

The clinical examination of both dogs was unremarkable. The female was on treatment for hypothyroidism with 300 μg of l-thyroxine twice daily (Forthyron 200; Eurovet Animal Health). A Schirmer tear test (Schirmer Tear Test; Schering-Plough Animal Health Corp.) was 22 and 20 mm/minute in the left and right eyes of the male dog and 25 and 21 mm/minute in the female dog, respectively. In the female, purulent conjunctivitis was diagnosed OS, with severely hyperaemic palpebral and bulbar conjunctivae covered with large lymphoid follicles. In the male, mild follicular hyperplasia was present on the bulbar aspect of the nictitating membrane OS. In both patients, four thread-like motile white parasites were observed in the conjunctival fornices OS (Fig 1). Fluorescein testing (Fluorescein; Haag-Streit International) was negative in both dogs. Bilateral nuclear sclerosis was present in both dogs. The male dog had a translucent iris cyst in the left anterior chamber. Bilateral small foci of retinal dysplasia in the tapetal area were present in the female with small posterior polar subcapsular lenticular opacities OS. The rest of the ocular examination was within normal limits in both dogs.

Treatment and outcome

The parasites were removed using fine serrated forceps and cotton tip applicators in both dogs. Topical anaesthesia with 4

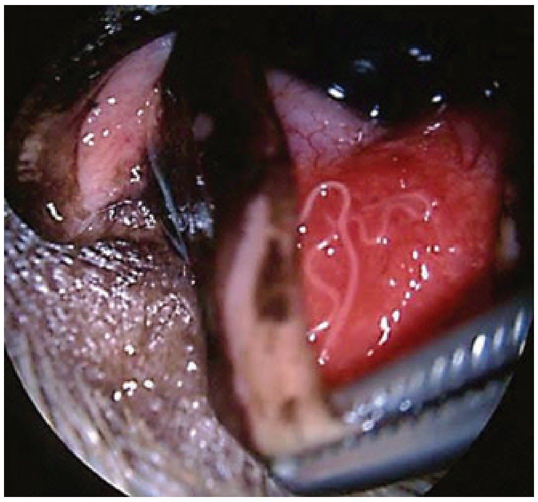

FIG 1. ***Thelazia callipaeda*** **on the inflamed conjunctival bulbar surface of the nictitating membrane**

mg/mL oxybuprocaine hydrochloride (0·5% Unicaïne; Thea Pharma) was instilled before parasite removal. The specimens were collected in 70% ethanol for parasitologic identification. Both patients were treated systemically with one dose of spot-on dermal application of 10% imidacloprid and 2·5% moxidectin (Advocate Spot-On; Bayer HealthCare) and topically with a 1 mg dexamethasone sodium phosphate and 4 mg/mL chloramphenicol (Deicol; Meda Pharma) solution thrice daily OS for 4 weeks.

Four weeks later, the infection had resolved in both dogs. No parasites were observed. However, in the female dog, mild follicular conjunctivitis persisted; therefore, the drops were continued for 2 further weeks. One month later, both owners reported by telephone that the eyes appeared normal.

Morphologic and molecular identification

Five female worms (15·5 ±2·5 mm long and 435 ±50 µm wide at the widest point) and one male worm (10·2 mm long and 360 µm wide at the widest point) were identified microscopically. All specimens were identified as *T. callipaeda* according to their size, the presence of a buccal capsule, the transversally striated cuticle, the position of the vulva located anterior to the oesophagus-intestinal junction and the presence of numerous rounded first-stage larvae in the distal uterus in the female worms and the presence of two dissimilar spicules in the caudal bursa of the male worm (Otranto and others 2003b) (Fig 2).

Molecular identification was performed with the worms collected as previously reported (Otranto and others 2005). Briefly, genomic DNA was isolated from the worms using QIAmp DNA Mini Kit (Qiagen GmbH). The cytochrome c oxidase subunit 1 (*cox1)* (689 bp) was amplified using described primers and a commercial kit (Taq PCR Master Mix; Qiagen GmbH). The amplification products were purified using a commercial kit (MSB® Spin PCRapace; Invitek) and sequenced with a genetic analyser (ABI PRISM® 3100; Applied Biosystem) and compared with the BLASTn genomic database (McGinnis and Madden 2004). The *cox1* sequences obtained were identical to the sequence representing haplotype 1 (h1) (GenBank accession number AM042549) (Otranto and others 2005).

DISCUSSION

Both dogs affected by ocular thelaziosis were living in Belgium when diagnosed, but had travelled and stayed in various regions of southern Europe. In a previous study, *T. callipaeda* was identified from a dog living in the Netherlands, which had spent 3 months in the Dordogne department (Otranto and others 2005). There is another report describing a case of canine Thelaziosis in Belgium but the information included is limited, although it was noted that the dog had travelled to the Lombardia region (Italy) (Janssens and Claerebout 2006). In western Europe, canine thelaziosis is now considered endemic and widespread in south-western France (Dordogne department, close to the Lot) (Dorchies and others 2007), in southern Switzerland and all of

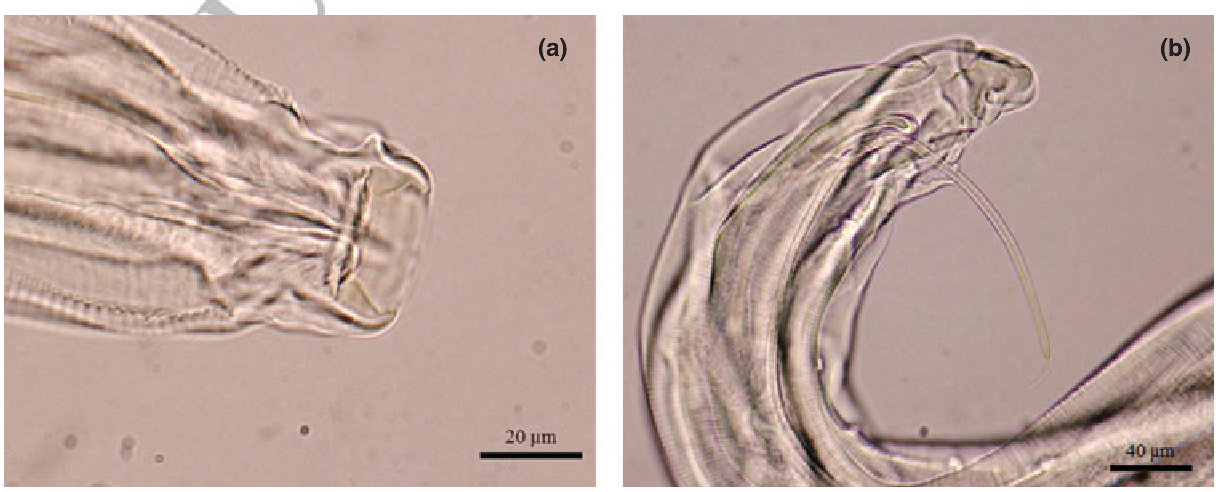

FIG 2. (a) Buccal capsule and transversally striated cuticle. (b) Male caudal bursa with unequal spicules

Italy, with a prevalence as high as 60% in the Basilicata region (Otranto and others 2003a).

The potential introduction and establishment of *T. callipaeda* in Belgium would depend on the presence of the fly vector. According to a previous study (Otranto and others 2006a) based on a predictive geoclimatic model, the vector, *P. variegata*, would be able to survive and multiply in Belgium. However, the presence of *P. variegata* has not yet been recorded in Belgium (Royal Belgian Institute of Natural History, http://www.species.be).

The disease can be subclinical or symptomatic, with 15·4 to 81·4% of infected dogs showing clinical signs (Malacrida and others 2008, Miro and others 2011). Affected dogs typically present with follicular conjunctivitis, a mucoid to purulent discharge and lymphoid tissue hyperplasia, as observed in the present cases (Ruytoor and others 2010). Conjunctival petechiae and oedema, epiphora (Miro and others 2011), keratitis and corneal ulcers are less frequently described (Dorchies and others 2007). Clinical signs may result from the mechanical damage to the ocular surfaces by the cuticle and parasite movement (Otranto 2011). The foreign body sensation can lead to self-mutilation and secondary infection of the eyelids, conjunctiva and cornea. Epiphora can result from nasolacrimal duct obstruction by the parasites (Janssens and Claerebout 2006). In dogs, the severity of symptoms did not appear to correlate with the number of worms found (Miro and others 2011). Because of the similarity in clinical signs, thelaziosis should be included in the differential diagnosis of infectious or allergic conjunctivitis, dacryocystitis and keratitis (Otranto 2011).

A diagnosis is made by finding the adult worms on the ocular surfaces, as observed in the present cases and/or in the nasolacrimal ducts. Diagnosis can be difficult when most parasites are in a larval stage, when few adult nematodes are present or when parasites are located within the excretory ducts of the lacrimal glands. The latter location has not been described in dogs to the author's knowledge. Identification of the worms can be performed by microscopic and molecular examination. Mitochondrial genes such as the *cox1* have proven useful for such investigations because of the relatively rapid evolutionary rates of these genes and the availability of gene sequences for filaroids in databanks (Hu and others 2004). In this study, the use of both techniques led to the identification of the same species: *T. callipaeda* (*cox1* h1).

Treatment of the condition is by removal of the worms. Topical corticosteroids and antibiotics can be used to treat the associated conjunctivitis and prevent bacterial contamination. A single dose of 10% imidacloprid and 2·5% moxidectin by spot-on dermal application (Advocate Spot-On®; Bayer) has previously been shown to be effective (Bianciardi and Otranto 2005, Janssens and Claerebout 2006). These treatment regimes were performed in the present cases. One percent moxidectin eye drops in an aqueous solution, administered as a single dose, was also highly efficient and well tolerated in infected dogs (Lia and others 2004).

In the southwest of France and the northwest of Italy, four cases of human *T. callipaeda* infection were diagnosed (Ruytoor and others 2010). Wild fauna, particularly red foxes and hares, probably plays a role in maintaining and spreading the nematode amongst humans and pets in rural areas (Ruytoor and others 2010). However, human thelaziosis is considered a neglected disease. This could be due to its high prevalence in socio-economically disadvantaged communities and the lack of awareness amongst physicians across Europe concerning the zoonotic potential of this parasite (Shen and others 2006).

To the authors' knowledge, this is the first case report of canine Thelaziosis in Belgium confirmed by microscopic and molecular identification. With the presence of definitive hosts and an increasing number of dogs travelling to and coming from southern endemic regions, the establishment of *T. callipaeda* in larger areas of Europe is possible. Further studies are required to explore the vectorial capacity of *Phortica* spp. in northern Europe, especially as the threat of global warming and climatic change increases.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. AQ1

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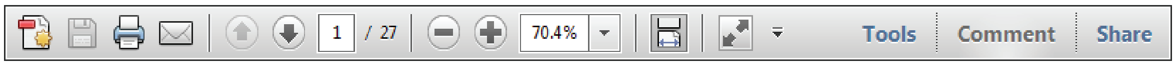

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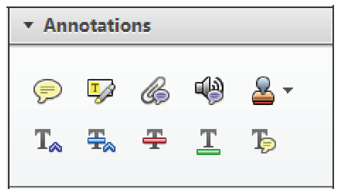

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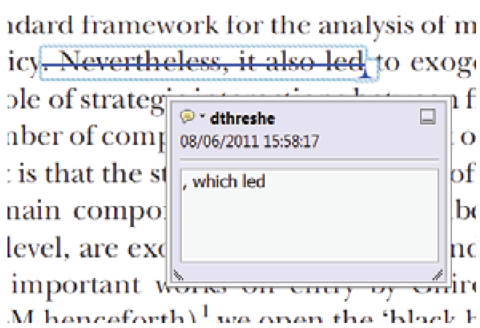

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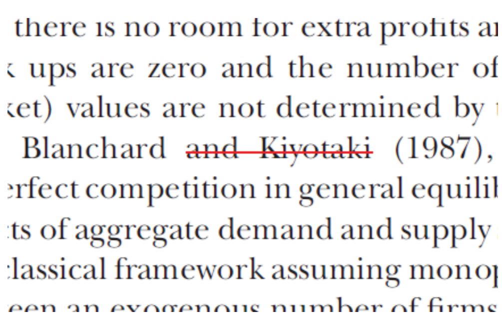

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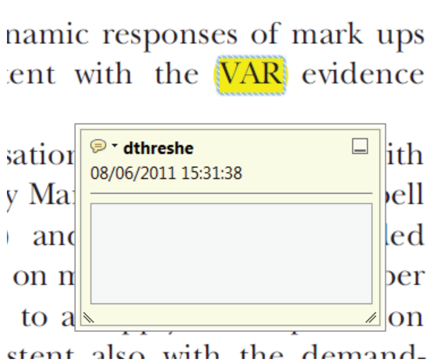

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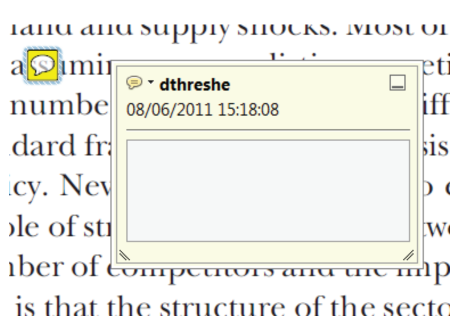

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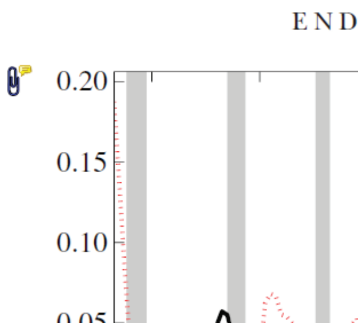

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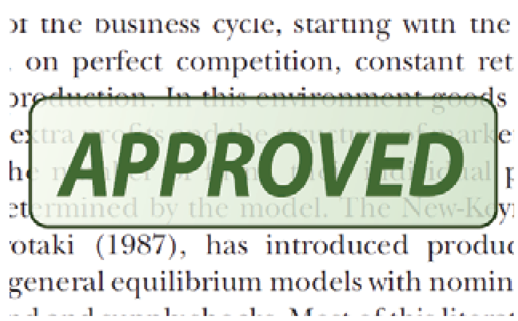

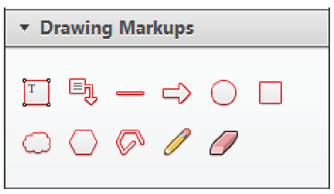

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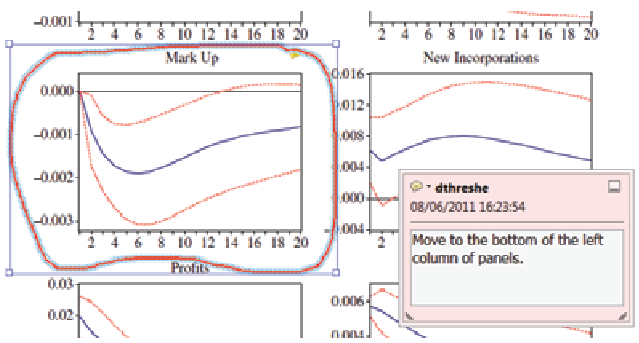

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