Veterinary Parasitology 252 (2018) 163–166

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

The eyeworm *Thelazia callipaeda* in Portugal: Current status of infection in pets and wild mammals and case report in a beech marten (*Martes foina*)

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ARTICLE INFO

Keywords:
Beech marten
Eyeworm
Martes foina
Portugal
Thelazia callipaeda
Wildlife diseases

ABSTRACT

Ocular thelaziosis is caused by nematodes of the genus *Thelazia* (Spirurida, Thelaziidae), which inhabit the surface of the eyes and associated tissues. *Thelazia callipaeda* affects a range of mammal species, including humans, and in the last two decades has been reported in multiple European countries, being classified as an emergent vector-borne pathogen. In Portugal *T. callipaeda* is endemic in north-eastern areas, where it has been reported in domestic dogs, cats, red foxes (*Vulpes vulpes*) and wild European rabbits (*Oryctolagus cuniculus*). We report, for the first time, *T. callipaeda* in a beech marten (*Martes foina*) from Portugal and highlight the presence of haplotype 1 as the only one found in Europe, irrespective of the host species and geographical area of provenience.

The genus *Thelazia* (Spirurida, Thelaziidae) includes a cosmopolitan group of eyeworms, some of them zoonotic, which are responsible for ocular infections in domestic and wild animals (Otranto and Traversa, 2005). At least 16 species have been identified in different hosts and, among them, *Thelazia callipaeda* Railliet and Henry, 1910 affects both animals and humans (Otranto and Eberhard, 2011).

Until two decades ago, *T. callipaeda* was almost exclusively reported in eastern countries, i.e. the former Soviet Republics, India, Thailand, Indonesia, Myanmar, Korea, China, Taiwan and Japan (Anderson, 2000; Shen et al., 2006; Colwell et al., 2011), thus being known as the "Oriental eyeworm". However, in the last few decades, *T. callipaeda* has increasingly been reported in both animals and humans in Europe, initially from Italy (Otranto et al., 2003a) and then from several other European countries, such as France (Dorchies et al., 2007), Switzerland (Malacrida et al., 2008), Germany (Magnis et al., 2010), Belgium (Caron et al., 2013), Spain (Fuentes et al., 2012; Miró et al., 2011), Bosnia and Herzegovina, Croatia (Hodžić et al., 2014), Serbia (Gajić et al., 2014), Romania (Mihalca et al., 2015), Hungary and Bulgaria (Colella et al., 2016) and Greece (Diakou et al., 2015; Papadopoulos et al., 2018). The increase in animal and human case reports makes this parasite an emergent vector-borne pathogen in Europe (Otranto et al., 2013).

In Portugal, *T. callipaeda* has been diagnosed in domestic dogs (Vieira et al., 2012; Pimenta et al., 2013; Maia et al., 2016), domestic cats (Rodrigues et al., 2012; Soares et al., 2013), red foxes (*Vulpes vulpes*) (Sargo et al., 2014) and wild European rabbits (*Oryctolagus cuniculus*) (Gama et al., 2016) (Table 1). Since the first reports of infection in dogs (Vieira et al., 2012), the number of cases has been increasing in northern and central regions of Portugal, especially in areas close to the Portuguese-Spanish border. Nowadays, *T. callipaeda* is endemic in north-eastern regions, at latitudes similar to those of other countries where human and animal infections are recognized to be endemic, such as Italy, India and Japan (Anderson, 2000; Otranto et al., 2003a, 2005b; Shen et al., 2006; Pimenta et al., 2013). In addition, *T. callipaeda* infection has been diagnosed in human patients from Spain, near the Portuguese-Spanish border (Fuentes et al., 2012; López Medrano et al., 2015), France, Italy (Otranto and Dutto, 2008), Croatia (Paradžik et al., 2016) and Serbia (Tasić-Otašević et al., 2016).

In wildlife, besides red foxes (Hodžić et al., 2014; Sargo et al., 2014) and rabbits (Otranto et al., 2004; Gama et al., 2016), *T. callipaeda* has been found in wolves (*Canis lupus*) (Otranto et al., 2009; Mihalca et al., 2016), golden jackals (*Canis aureus*) (Mihalca et al., 2016), brown hares

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https://doi.org/10.1016/j.vetpar.2018.02.007
Received 28 December 2017; Accepted 2 February 2018

Table 1
Data on 44 cases of infection with the eyeworm *Thelazia callipaeda* in vertebrate hosts from Portugal.

Host	No. of infected hosts	No. of worms per host (mean)	Clinical manifestations[a]	Geographical area	Host aptitude/condition	Reference
Dog (*Canis familiaris*)	9	17.9	Ocular discharge, conjunctivitis and ocular pruritus	North-eastern	Guard, hunting and pet	Vieira et al. (2012)
	1	8	Ocular pain, conjunctival hyperemia with serous discharge and blepharitis	North-central	Pet	Pimenta et al. (2013)
	22[b]	8.1	Conjunctivitis, epiphora, purulent discharge, keratitis, uveitis, blepharitis, photophobia and ulceration	Centre-eastern	Guard, hunting, pastoral and pet	Maia et al. (2016)
Cat (*Felis catus*)	1	7	Infra-orbital abscess, chronic conjunctivitis and oedema of the conjunctiva	North-eastern	Pet	Rodrigues et al. (2012)
	1	16	Ocular discharge, blepharospasm, photophobia, purulent secretions and mild conjunctival edema	Centre	Pet	Soares et al. (2013)
	4[c]	4.3	Conjunctivitis	Centre-eastern	Pet	Maia et al. (2016)
Red fox (*Vulpes vulpes*)	3[d]	5.7	Exudative conjunctivitis	Northern (= 1) and Centre-eastern (n = 2)	Trapped (n = 1) or legally shot (n = 2)	Sargo et al. (2014)
Wild European rabbit (*Oryctolagus cuniculus*)	2	2.0	None[e]	North-eastern	Necropsy (haemorrhagic viral disease)	Gama et al. (2016)
Beech marten (*Martes foina*)	1	1	None[e]	North-central	Necropsy	The present report

[a] Observed in at least one infected host.
[b] Representing 3.8% of 586 dogs; the percentage of infected dogs with ocular manifestations was 45.5% (10/22).
[c] Representing 23.5% of 17 cats; the percentage of infected cats with ocular manifestations was 25% (1/4).
[d] The percentage of infected red foxes with ocular manifestations was 33.3% (1/3).
[e] No lesion compatible with ocular thelaziosis.

(*Lepus europaeus*), beech martens (*Martes foina*) (Otranto et al., 2009) and wildcats (*Felis silvestris*) (Otranto et al., 2009; Mihalca et al., 2016). However, as the number of reports from wild carnivores is still scarce, there is little information on the role of these species as reservoir hosts of this nematode.

The beech marten *M. foina* (Carnivora, Mustelidae), also known as stone marten, has a wide geographical distribution in Europe, occurring in urban and suburban areas. This species is classified as of “Least Concern” status in the Portuguese Red Data Book, as well as in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, due to its wide distribution and large population (Cabral et al., 2006). Wild carnivores can carry zoonotic pathogens, including parasites such as *Toxoplasma gondii*, *Echinococcus multilocularis*, *Taenia* spp., *Toxocara canis* and ancylostomatids, therefore acting as potential spreaders and contributing to animal-to-animal spill-over of diseases, as well as to transmission to humans (Lempp et al., 2017). Though *T. callipaeda* infection has been described in beech martens in Italy (Otranto et al., 2009), no further reports of ocular infection in this host species are available from other countries including Portugal.

The corpse of a female beech marten killed on the road was found near Vila Real (41°19′12.59″N; 7°38′46.82″W), in north-central Portugal. The animal was frozen and submitted to *post mortem* examination at the Laboratory of Histology and Pathology of the University of Trás-os-Montes e Alto Douro (UTAD). Necropsy examination revealed multiple bone and organ fractures and internal haemorrhages, multiple cranial fractures and enucleation of the right eye. In the left eye, a filiform worm was noticed in the conjunctival sac (Fig. 1), but no clear ocular lesions were observed (Table 1).

The worm was collected and stored in 70% ethanol. Morphological identification was done at the Laboratory of Parasitology of UTAD according to the keys proposed by Skrjabin et al. (1967) and Otranto et al.

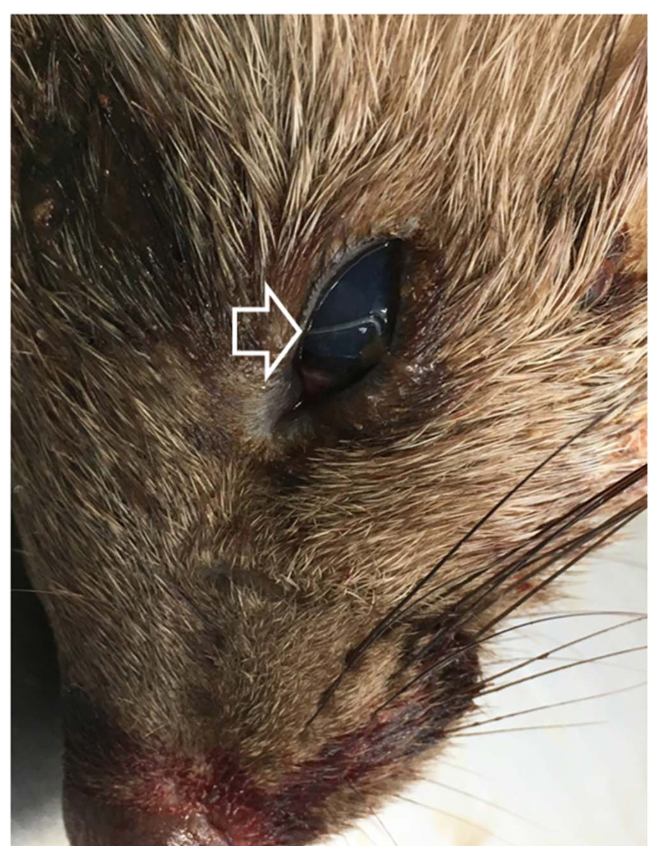

Fig. 1. Left eye of beech marten.*Thelazia callipaeda* adult worm (arrow).

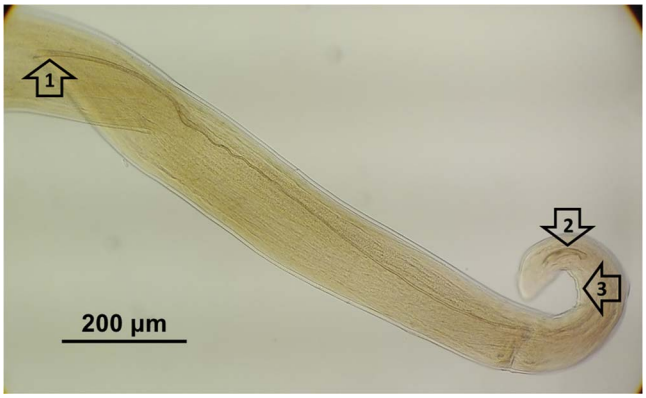

Fig. 2. Adult male *Thelazia callipaeda*. Posterior end with long spicule (arrow 1), short spicule (arrow 2) and post-cloacal papillae (arrow 3).

Fig. 3. Adult male *Thelazia callipaeda*. Anterior extremity with buccal capsule, and transverse striations over the tegumental surface.

(2003b). The male worm was morphologically characterized on the basis of the pre-cloacal and post-cloacal papillae and length of the left spicule, which is much longer (about 10 times) than the right one (Fig. 2); a buccal capsule and transverse striations over the tegumental surface were also observed (Fig. 3), leading all these features together to the identification of *T. callipaeda*.

Molecular identification was carried out as previously described (Otranto et al., 2005b), by PCR amplification of partial mitochondrial cytochrome c oxidase subunit 1 gene (*cox*1, 689 bp). Sequences obtained were compared with those available in GenBanK dataset by Basic Local Alignment Search Tool (BLAST) analysis, indicating a 100% identity to the sequence of *T. callipaeda* haplotype h1 (GenBank accession number AP017700).

The infected beach marten was found in the same geographical area where thelaziosis was previously diagnosed in domestic and wild mammals, such as dogs, cats, red foxes and wild rabbits (Vieira et al., 2012; Pimenta et al., 2013; Sargo et al., 2014; Gama et al., 2016), demonstrating that this geographical area provides suitable habitats for the worm to complete its life cycle when the vector is present. Indeed, *T. callipaeda* is transmitted in Italy by a drosophilid insect, *Phortica variegata* (Diptera, Steganinae) (Otranto et al., 2005a, 2006). An ecological niche model suggested that vast areas of Europe were suitable for the development of *P. variegata* (Otranto et al., 2006), foreseeing the current spread of *T. callipaeda* to previously non-endemic areas. However, that model did not consider the role of wild carnivores as spreaders of the infection in new areas in which the vector was present. Indeed, the transmission of *T. callipaeda* occurs when the vector insects feed on lachrymal secretions, depositing third-stage larvae that develop into adult worms in the vertebrate hosts (Otranto et al., 2006). *T. callipaeda* may cause a range of clinical manifestations, such as epiphora, photophobia, keratitis, conjunctivitis, which may hamper the vision of affected animals (Otranto and Traversa, 2005; Otranto et al., 2009; Gama et al., 2016). The animal reported in this study had no detectable ocular alterations or eye surface disease. Clinical manifestations of mild to serious ocular disease due to the presence of *T. callipaeda* have been previously reported (Maia et al., 2016), but parasites have frequently been identified in apparently healthy animals (Miró et al., 2011).

Otranto et al. (2007) suggested that wild animals may act as reservoirs of infection for domestic species. Most of the data on *T. callipaeda* infecting wildlife in Europe refers to red foxes, which are considered a presumptive reservoir, as they have been found infected, with high prevalence, in areas where thelaziosis has also been reported in dogs (Otranto et al., 2009; Sargo et al., 2014). However, other wild carnivores can also be natural hosts for *T. callipaeda* and the present report increases the range of definitive hosts of the eyeworm in Portugal and southern Europe. Being a common species, the beech marten may play a role in maintaining and spreading the nematode amongst pets and humans in rural areas (Otranto et al., 2007; Ruytoor et al., 2010; Hodžić et al., 2014; Sargo et al., 2014). Despite this potential role of wild fauna in spreading *T. callipaeda*, the presence of thelaziosis in Europe might also be a consequence of the increased mobility of humans and pets, and of vector distribution (Otranto et al., 2003a; Colwell et al., 2011; Miró et al., 2011). Several descriptions suggest that a sylvatic cycle of thelaziosis occurs under natural conditions most likely due to the contact of wildlife with the vector (Miró et al., 2011; Hodžić et al., 2014).

In conclusion, the present study extends the host spectrum of *T. callipaeda* in Portugal, emphasizing the potential role of wild carnivores as reservoirs of this zoonotic nematode. Further studies on domestic and sylvatic cycles of *T. callipaeda*, as well as their intersection, are crucial to the knowledge of the ecology, epidemiology and pathology of ocular thelaziosis.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

All the procedures in this study were in accordance with the Portuguese legislation for the protection of animals (Decree-Law nº 113/2013).

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