

Short communication

First report of human *Thelazia callipaeda* infection in Portugal

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ABSTRACT

The zoonotic parasitic nematode *Thelazia callipaeda*, also known as the oriental eye worm, is endemic in several European countries, including Portugal. Infections may result in ocular disease in domestic and wild animals as well as humans, with more or less severe manifestations. We report the first human case of ocular thelaziosis by *T. callipaeda* in Portugal, a country where the parasite had already been found to infect dogs, cats, red foxes, wild rabbits and a beech marten. An 80-year-old patient from east-central Portugal, who had been suffering from tearing for a few years, had whitish filiform fragments removed from the left eye. Polymerase chain reaction of partial cytochrome c oxidase subunit 1 and 18S small subunit rRNA genes followed by bidirectional sequencing and BLAST analysis confirmed *T. callipaeda* haplotype 1, the only haplotype previously reported in Europe. The endemicity of *T. callipaeda* in domestic and wild animals in east-central Portugal makes it very likely that infection of the human patient had occurred locally. In east-central and other geographical areas of Portugal, veterinarians and physicians, especially ophthalmologists, should regard *T. callipaeda* as a cause of ocular pathology in animals and humans.

Thelazia callipaeda (order Spirurida; family Thelaziidae), also known as the oriental eye worm, is a nematode living on the conjunctival surfaces of domestic and wild animals as well as humans (do Vale et al., 2019). This zoonotic parasite and the disease it causes, ocular thelaziosis (or thelaziasis), are endemic in Asian and European countries (Otranto et al., 2020). In Europe, under natural conditions, the life cycle of *T. callipaeda* comprises the fruit fly *Phortica variegata* (order Diptera; family Drosophilidae) as intermediate host and vector. Male specimens of this insect ingest first-stage larvae while feeding on lachrymal secretions of infected mammals (definitive hosts) and transmit infective third-stage larvae later on (Otranto et al., 2006; Otranto and Dantas-Torres, 2015). Infections with *T. callipaeda* may be subclinical or asymptomatic, while clinical signs of ocular thelaziosis, both in animals and humans, include blepharospasm, conjunctivitis, tearing (or epiphora), itching, ocular discharge and even keratitis and eye ulceration

(do Vale et al., 2019; Otranto et al., 2020).

The presence of *T. callipaeda* in Europe was first reported in dogs from Piedmont, northwestern Italy, near the end of the 20th century (Rossi and Bertaglia, 1989). Since then, the parasite was confirmed throughout several other European countries, viz. (in order of appearance) France, Germany, Switzerland, Spain, Portugal, Belgium, Bosnia and Herzegovina, Croatia, Serbia, Romania, Greece, Bulgaria, Hungary, Slovakia, the United Kingdom, Turkey, Austria, Czech Republic, Moldova and Poland (do Vale et al., 2019, 2020; Rolbiecki et al., 2021). In Portugal, *T. callipaeda* has already been found to infect dogs (Vieira et al., 2012; Pimenta et al., 2013), cats (Rodrigues et al., 2012; Soares et al., 2013), red foxes (*Vulpes vulpes*; Sargo et al., 2014), wild rabbits (*Oryctolagus cuniculus*; Gama et al., 2016) and a beech marten (*Martes foina*; Seixas et al., 2018), but not humans, so far.

This report describes the first human case of ocular thelaziosis by

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T. callipaeda in Portugal. The work reported has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and informed consent was obtained from the patient. An 80-year-old patient living in the municipality of Guarda, in east-central Portugal, who had suffered from tearing for some years, underwent cataract surgery in the left eye in October 2021. Within the scope of the surgical procedure, whitish and mobile filiform fragments were removed from the conjunctival cul-de-sac and later identified as “filaria-type parasitic agents”. Nevertheless, a complementary microscopic observation raised the morphological suspicion of *Thelazia* spp. worms (Fig. 1). Following worm extraction, the patient received a prescription of ivermectin (200 µg/kg, *per os*, repeated after 7 days).

A parasite sample was subjected to molecular analysis for species confirmation and a fragment was initially processed as previously described (Otranto et al., 2005), followed by nucleic acid extraction with a customized version of the QIAamp® DNA Mini Kit (Qiagen Inc, Valencia, CA, United States of America) on an automated platform (QIAcube, Qiagen GmbH, Germany). Parasite DNA detection was performed using previously described assays targeting the cytochrome c oxidase subunit 1 (*cox1*; 689 bp) (Otranto et al., 2005) and the 18S small subunit rRNA genes (18S rRNA; 900 bp) (Floyd et al., 2005). Nucleic acid amplifications were carried out using Xpert Fast Hotstart Mastermix (2X) with dye (GRiSP, Porto, Portugal), according to the company’s protocol instructions. Briefly, amplification was carried out on 25 µL reaction mix containing 1 µL of each mentioned primer at 10 pmol/µL, 12.5 µL of Xpert Fast Hotstart Mastermix (2X) with dye, 5.5 µL PCR grade water and 5 µL of extracted DNA. Amplified products of the expected size were purified with GRS PCR & Gel Band Purification Kit (GRiSP, Porto, Portugal) and bidirectionally sequenced. Consensus sequences were compared with the sequences available in the NCBI (GenBank) nucleotide database (<http://blast.ncbi.nlm.nih.gov/Blast>). Sequence analysis with Basic Local Alignment Search Tool (BLAST) of the *cox1* gene revealed 100% identity with *T. callipaeda* haplotype 1 (accession number AM042549.1), the only haplotype previously reported in Portugal (Seixas et al., 2018) and Europe (do Vale et al., 2019). Sequence analysis of the 18S rRNA gene also revealed 100% identity with *T. callipaeda* (accession number MW570771). The sequences of *T. callipaeda* detected in this study are available at GenBank under accession numbers OM470911 (*cox1*) and OM469330 (18S rRNA).

This first human case of ocular thelaziosis by *T. callipaeda* in Portugal is reported from east-central Portugal, a geographical area where the parasite was previously found in red foxes (Sargo et al., 2014), dogs and cats (Maia et al., 2016). In the contiguous municipalities of Guarda (where the human patient lived), Gouveia, Sabugal, Belmonte, Covilhã, Penamacor and Fundão, encompassing 3773.68 square km (40°39048’N to 40°03004’N and 06°48045’W to 07°48003’W) and with an altitudinal range of <500–1100 m above sea level, 22 (3.8%) of 586 dogs and four (23.5%) of 17 cats were found infected with *T. callipaeda*. Out of the 22 infected dogs, 10 (45.4%) had clinical thelaziosis (Maia et al., 2016). These facts support *T. callipaeda* endemicity in east-central Portugal and, together with the circumstance that the patient had not travelled out of the area for approximately 1.5 years before worm extraction, make it very likely that infection had occurred locally. The patient had regular contact with a young dog with outdoor access, but this animal was not found to be infected with *T. callipaeda*.

The two detected fragments of *T. callipaeda* were from female worms, which had larvae inside them (Fig. 1). These larvae may also mean that males were present on the conjunctival sac of the patient and the same might be true for additional female worms. The administration of ivermectin, an effective nematocide, has most probably resolved infection in the medium term, as a follow-up examination of the patient did not reveal any additional eye worms. In animals, macrocyclic lactones, such as moxidectin, can be used to treat infections with *T. callipaeda* and also have a prophylactic effect (Marino et al., 2021).

In areas of endemicity, such as east-central Portugal, infection with *T. callipaeda* should be included in the differential diagnosis of clinical

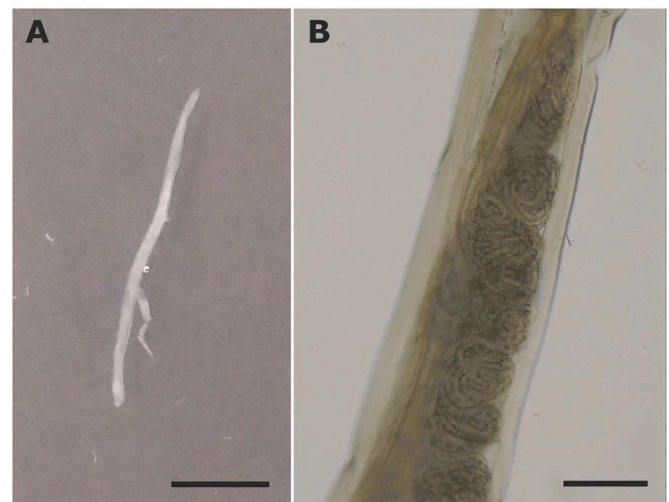


Fig. 1. (A) Fragment of a female *Thelazia callipaeda* worm; bar = 2 mm. (B) Central portion of the *T. callipaeda* female fragment with coiled first-stage larvae; bar = 200 µm.

conditions comprising blepharospasm, conjunctivitis, tearing (or epiphora), itching, ocular discharge, keratitis and eye ulceration. From a One Health perspective, this approach should apply to humans, domestic and wild animals.

In conclusion, *T. callipaeda* is endemic in east-central Portugal where it has already infected domestic (dogs and cats) and wild carnivores (red foxes) and now also a human patient. A potentially close contact between infected hosts – vertebrates and *P. variegata* – contributes to promote a zoonotic circulation of *T. callipaeda*. In east-central Portugal and other geographical areas of the country, this emerging zoonotic parasite should be regarded by veterinarians and physicians, especially ophthalmologists, as a cause of ocular pathology in animals and humans.

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Authors contribution statement

António Carlos Tomé Morgado: conceptualization, investigation, data curation. Beatriz do Vale: formal analysis, writing – review and editing. Paulo Ribeiro: investigation, resources. Teresa Coutinho: investigation, visualization. Sérgio Santos-Silva: investigation. Alcía de Sousa Moreira: investigation. Filipa T. Rodrigues: visualization. Ana Cláudia Coelho: writing – review and editing, funding acquisition. Ana Patrícia Lopes: writing – review and editing, funding acquisition. João R. Mesquita: methodology, writing – review and editing, funding acquisition. Luís Cardoso: writing – original draft, supervision. All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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