



RAPID COMMUNICATION

Zoonotic *Thelazia callipaeda* eyeworm in brown bears (*Ursus arctos*): A new host record in Europe

Elias Papadopoulos¹  | Anastasia Komnenou¹ | Alexandros A. Karamanlidis^{2,3} | Marcos Antonio Bezerra-Santos⁴  | Domenico Otranto^{4,5} 

¹ School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki Greece, Thessaloniki, Greece

² ARCTUROS, Civil Society for the Protection and Management of Wildlife and the Natural Environment, Florina, Greece

³ Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway

⁴ Department of Veterinary Medicine, Università degli Studi di Bari, Bari, Italy

⁵ Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran

Correspondence

Domenico Otranto, Department of Veterinary Medicine, University of Bari, Valenzano, 70010 Bari, Italy.

Email: domenico.otranto@uniba.it

Abstract

Thelazia callipaeda (Spirurida, Thelaziidae) eyeworm, a zoonotic parasite transmitted by lachryphagus drosophilids, causes subclinical to clinical ocular disease in a wide range of vertebrates, including humans. In the past 20 years, the infection spread primarily in areas where the vector thrives, and now is endemic in many European countries. Recently, this nematode has been reported also in the United States. Here, we describe the first record of the brown bear (*Ursus arctos*) as a host of *T. callipaeda*. Eight (26.7%) out of 30 bears were found to be infected with *T. callipaeda* adult worms. In addition, the parasite was detected in 13 wolves (*Canis lupus*) sharing the same environment with the brown bears. At the molecular characterization, all nematodes belonged to the haplotype 1, which is the only one recorded in Europe, as yet. This finding demonstrates that brown bears may act as hosts for this parasite, suggesting its potential role in the maintenance of the cycle not only in the wild but also in captivity. Under the above circumstances, the brown bear may represent a potential zoonotic risk for humans, both in rural and urban areas where other reservoirs may be infected.

KEYWORDS

brown bear, eyeworm, Greece, *Phortica*, *Thelazia callipaeda*, *Ursus arctos*, wolf, zoonotic parasite

1 | INTRODUCTION

The zoonotic eyeworm, *Thelazia callipaeda* (Spirurida: Thelaziidae) is commonly found on the conjunctiva, under the lids, the nictitating membrane and other surrounding ocular tissues of several vertebrate hosts, including humans (Otranto et al., 2021). This parasite is transmitted by the zoophilic fruit fly *Phortica variegata* (Drosophilidae: Steganinae) (Otranto et al., 2009), a lachryphagous drosophilid that feeds on ocular secretions of receptive hosts transmitting infective third stage larvae (L3) during the warmer months of the year (i.e. from April to October in Europe) (Otranto et al., 2006). The epidemiology of *T. callipaeda* is strictly related to the occurrence of domestic and wild reservoirs, as well as competent vectors, which play a relevant role in the maintenance and spreading of the parasite into previously non-endemic regions (Otranto et al., 2007, 2009).

The brown bear (*Ursus arctos*) is one of the most endangered vertebrates in Greece. Two segregated populations exist in the northeastern

and western parts of Greece, with an estimated 450 individuals living in the country, and a recent increasing trend in the population size (Karamanlidis et al., 2015). In order to investigate brown bears as intermediate hosts for *T. callipaeda*, we examined individuals from an endemic area of Greece and reported them as a new host for this parasite.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

Samples were collected from bears and wolves (*Canis lupus*) hosted at the sanctuaries of the environmental organization ARCTUROS (<https://www.arcturos.gr/en/>). The sanctuaries are located in the Prefecture of Florina, Northern Greece (latitude 40.652 and longitude 21.503, 1353 m above sea level). In addition, free-ranging animals that died by car accidents were also sampled.

TABLE 1 Individual animal details, eyeworm numbers and symptoms of the brown bears found infested with *Thelazia callipaeda*

Individual	Sex	Age (years)	Number of recovered worms (male/female)	Symptoms
1	M	5	11 (3/8)	Conjunctivitis, oedema, epiphora and petechiae
2	F	4	3 (2/1)	Ocular discharge
3	M	2	4 (1/3)	None
4	F	1	5 (3/2)	Keratitis
5	F	1	7 (3/4)	Ocular discharge
6	M	1	2 (2/0)	None
7	M	1	3 (1/2)	None
8	M	1	5 (4/1)	Mild conjunctivitis

**FIGURE 1** Presence of *Thelazia callipaeda* adult worms in the eyes of brown bears from Greece. (a) Lateral view; (b) and (c) medial view with ocular discharge; (d) keratitis and ocular discharge

Thirty brown bears, from which two (cases 1 and 2) were free-ranging, and the remaining hosted in a fenced area of the ARCTUROS Brown Bear Sanctuary, were examined for the presence of eyeworms. In addition, 13 wolves were also evaluated (i.e. 12 hosted in the same sanctuary and one free-ranging). All alive animals were examined after sedation during their translocation within the ARCTUROS premises, or during necropsy of free-ranging animals that died in road accidents (two bears, and one wolf). At the ocular examination, nematode parasites (eyeworms) were collected from the eyes using sterile cotton swabs, forceps and flushing with physiological saline solution. The nematodes were placed into vials containing ethanol for further morphological and molecular identification.

2.2 | Morphological and molecular analysis

All nematodes were identified using standard morphological identification keys (Otranto, Lia, et al., 2003). For molecular confirmation, genomic DNA of individual worms was extracted using Dneasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Thereafter, PCR analy-

sis was performed using forward NTF (5'-TGATTGGTGGTTTGGTAA-3') and reverse NTR (5'-ATAAGTACGAGTATCAATATC-3') primers that amplify a portion (689 bp) of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene. PCR products were purified and sequenced in both directions using the Big Dye Terminator v.3.1 chemistry in a 3130 Genetic analyzer (Applied Biosystems, California, USA) in an automated sequencer (ABI-PRISM 377). Sequences were analyzed using MEGA7 software and compared with sequences available in GenBank through the BLAST search tool.

3 | RESULTS AND DISCUSSION

Nematodes were detected in the conjunctival sac of the eyes of 26.7% (8/30) of brown bears (Table 1; Figure 1), and 100% (13/13) of wolves examined (Table 2). All the specimens collected were morphologically identified as *T. callipaeda* and confirmed by nucleotide sequencing of a portion of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene. Sequences presented 100% nucleotide identity with *T. callipaeda* haplotype 1 available in GenBank (AM042549.1), which was previously

TABLE 2 Individual animal details, eyeworm numbers and symptoms of the wolves found infested with *Thelazia callipaeda*

Individual	Sex	Age (years)	Number of recovered worms (male/female)	Symptoms
1	M	3	9 (3/6)	Mucus, corneal ulcers, mucopurulent discharge, red eye
2	M	3	10 (3/7)	Blepharospasm, mucopurulent discharge, red eye, corneal ulcers
3	F	5	7 (4/3)	Blepharospasm, red eye, mucopurulent discharge
4	M	3	16 (6/10)	Blepharospasm, mucopurulent discharge, red eye, mild cornea oedema
5	F	5	13 (4/9)	Blepharospasm, mucopurulent discharge, red eye
6	F	6	6 (2/4)	Mucus, mucopurulent discharge, red eye, mild cornea oedema
7	F	6	8 (3/5)	Mucus, mucopurulent discharge, red eye
8	M	4	13 (5/8)	Blepharospasm, mucopurulent discharge, red eye, mild cornea oedema
9	M	5	10 (1/9)	Mucus, mucopurulent discharge, red eye
10	F	4	15 (4/11)	Mucus, mucopurulent discharge, red eye, mild cornea oedema
11	M	6	8 (4/4)	Blepharospasm, mucopurulent discharge, red eye, mild cornea oedema
12	F	5	7 (2/5)	Blepharospasm, mucopurulent discharge, red eye, mild cornea oedema
13	F	6	9 (1/8)	Blepharospasm, mucopurulent discharge, red eye

reported in all cases described in Europe. The obtained nucleotide sequences were submitted to GenBank under the accession number OK662943.

This study reports for the first time the occurrence of ocular thelaziosis by *T. callipaeda* in brown bears, broadening the host range of this nematode. In addition, grey wolves living in close proximity to the bears were also found to host this parasite, indicating the spread of *T. callipaeda* among different wildlife species. To the best of our knowledge, this parasite has not been recorded in bears in Europe, and in Asia a single case has been recently reported in a black bear from a natural reserve in China (Jin et al., 2021). This vector-borne nematode is of increasing importance due to its veterinary and public health implications (do Vale et al., 2019). Since the first reports in Italy (Otranto, Ferroglio, et al., 2003; Rossi & Peruccio, 1989), an increasing presence of autochthonous cases of thelaziosis by *T. callipaeda* have been recorded in Europe, such as in Austria (Hodžić et al., 2019), Belgium (Caron et al., 2013), France (Dorchies et al., 2007), Switzerland

(Malacrida et al., 2008), Spain (Miró et al., 2011; Marino et al., 2018), Portugal (Vieira et al., 2012), Romania (Mihalca et al., 2015), Hungary (Farkas et al., 2018) and Greece (Papadopoulos et al., 2018). More precisely in Greece, *T. callipaeda* is widespread in the northern and central parts of the country, with records in dogs, cats and in a rabbit (Papadopoulos et al., 2018). The fact that veterinary practitioners are becoming more familiar with the presence of this parasite might have contributed to new records. However, the rather apparent clinical presentation (i.e. mild conjunctivitis, follicular hypertrophy of the conjunctiva, foreign body sensation, epiphora, itchiness, congestion, swelling, hypersensitivity to light and keratitis) renders the diagnosis of thelaziosis not so difficult, suggesting the recent expansion of *T. callipaeda* in some areas. Similar to other vertebrate hosts, both animal species herein evaluated presented ocular clinical signs associated with *T. callipaeda* infection, with potential implication on the health and welfare of affected individuals. It seems that although there is a high degree of specificity of *T. callipaeda* for their vectors, there is not for their

definitive hosts (Otranto et al., 2006). The existence of a sylvatic life cycle of *T. callipaeda* has been proven in foxes in areas with high prevalence of canine thelaziosis, thus indicating that foxes act as one of the main reservoirs of *T. callipaeda* where pet animals are under veterinary control (Otranto, Lia, et al., 2003; Rossi et al., 2002). This could also account for the increasing number of reports of canine thelaziosis from different European areas over the past few years (Otranto, Lia, et al., 2003).

The cases of the infected brown bears in Greece come from both free-ranging animals and animals held in captivity. These individuals shared the same environment with wolves that were also infected with eyeworms. In all cases, the close vicinity of brown bears to wolves suggests these canids as the possible source of infection. This is also the case in other previously non-endemic European countries, where the geographical dispersion of *T. callipaeda* was attributed to several wildlife species, such as wild carnivores (e.g. red foxes, beech martens, wolves and badgers) and lagomorphs (e.g. hares and wild rabbits) (Dumitrache et al., 2018; Gama et al., 2016; Ionică et al., 2019; Mihalca et al., 2016; Otranto et al., 2007, 2009; Otranto & Dantas-Torres, 2015). This is further supported by the cases in areas with no reports of infested domestic animals, where the role of wild carnivores in introducing, maintaining and spreading this nematode was crucial (Otranto & Dantas-Torres, 2015). In addition, the role of wildlife trade could be a route for the spread of this parasite, as suggested for other parasites of zoonotic concern (Bezerra-Santos et al., 2021a, 2021b).

Findings herein reported are of public health concern, as captive bears kept in zoological gardens might act as a source of infection by *T. callipaeda* to humans visiting these enclosures. For example, bears kept in zoological gardens from China have been suggested as a possible risk factor to human infection with zoonotic parasites such as *Cryptosporidium* spp. and *Enterocytozoon bieneusi* (Wang et al., 2020). In addition, the role of free-ranging brown bears as spreaders of this parasite to humans and domestic animals should also be considered, mainly regarding individuals (e.g. farmers, hunters, hunting dogs and livestock) living in close contact with this wildlife species.

Data herein reported contribute to the epidemiological map of thelaziosis by *T. callipaeda* in Europe by adding brown bears as a new host species for this parasite. This finding further confirms the wide host range of this parasite, as well as its importance for veterinary and public health, as it also indicates the potential zoonotic risk for humans living in rural areas (Baneth et al., 2016). Therefore, monitoring and controlling this parasite in endemic areas should be considered to diminish its impact on wildlife, domestic hosts and humans.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page have been adhered to and all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The operation of the ARCTUROS Wolf and Bear Sanctuaries has been authorized by the special permit of the Hellenic Ministry of Agriculture 115130/6212. All research activities of ARCTUROS (e.g. necropsies) have been authorized by the special research permits of the Hellenic Ministry of Environment and Energy 135974/261, 178102/67 and 770/18.

AUTHOR CONTRIBUTIONS

Conceptualization, investigation, formal analysis and writing original draft: Elias Papadopoulos. *Investigation, methodology, formal analysis and writing original draft:* Anastasia Komnenou. *Writing: review and editing and formal analysis:* Marcos Antonio Bezerra-Santos. *Investigation, methodology and writing original draft:* Alexandros A. Karamanlidis. *Validation, supervision, writing: review and editing:* Domenico Otranto.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Elias Papadopoulos  <https://orcid.org/0000-0003-2564-5203>

Marcos Antonio Bezerra-Santos  <https://orcid.org/0000-0003-0575-6758>

Domenico Otranto  <https://orcid.org/0000-0002-7518-476X>

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