



# First report of eyeworm infection by *Thelazia callipaeda* in gray wolf (*Canis lupus*) from Serbia

Gajić Bojan<sup>1</sup> · Bugarski-Stanojević Vanja<sup>2</sup> · Penezić Aleksandra<sup>3</sup> · Kuručki Milica<sup>3</sup> · Bogdanović Neda<sup>3</sup> · Ćirović Duško<sup>3</sup>

Received: 6 May 2019 / Accepted: 22 October 2019 / Published online: 13 November 2019  
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

## Abstract

*Thelazia callipaeda*, originally known as an “Oriental eyeworm,” is a small nematode parasitizing the conjunctival sacs of domestic and wild animals and humans. Previous studies conducted in Serbia have reported the eyeworm infections in dogs, cats, and foxes, as well as in a human patient. As the data regarding thelaziosis from wildlife is still scarce, the aim of this study was to investigate the presence of *T. callipaeda* in gray wolf (*Canis lupus*) from Serbia. All collected nematodes were morphologically identified as *T. callipaeda* males ( $n = 64$ ) or females ( $n = 225$ ). Molecular characterization, conducted by PCR amplification followed by sequence analysis of partial cytochrome *c* oxidase subunit 1 gene (*cox1*), revealed only haplotype 1 of *T. callipaeda*. The overall prevalence of thelaziosis was 38.1% (8/21). In all positive animals, both eyes were affected, with a total parasitic load ranging from four to 132 worms per animal. Our results indicate the important epidemiological role of wolves as wildlife reservoirs of *T. callipaeda*, expanding geographic range of infection, as well as intra- and interspecies contact rates, although the role of other wild carnivore species (i.e., foxes and jackals) should be investigated in future studies.

**Keywords** Cox1 · h1 haplotype · Reservoir · Thelaziosis · Wildlife

## Introduction

*Thelazia callipaeda* (Spirurida, Thelaziidae) is an insect-borne nematode living in the conjunctival sac of domestic and wild carnivores, rabbits, and humans (Anderson 2000). Regardless of the definitive host species, the only confirmed vector for *T. callipaeda* under natural conditions in Europe is a zoophilic fruitfly *Phortica variegata* (Drosophilidae, Steganinae) (Otranto et al. 2006). The nematode was traditionally known as “Oriental eyeworm” due to its occurrences in some Asian countries and the former Soviet Union (summarized in Otranto et al. 2009).

After the first detection in dogs from Northern Italy (Rossi and Bertaglia 1989), *T. callipaeda* has been increasingly reported in dogs and cats from other European countries including France (Dorchies et al. 2007), Switzerland (Malacrida et al. 2008), Germany (Magnis et al. 2010), Spain (Miró et al. 2011), and Portugal (Vieira et al. 2012). More recent findings indicate that thelaziosis has been spreading in both domestic and wild carnivores across the Balkan countries (Colella et al. 2016), such as Bosnia and Herzegovina and Croatia (Hodžić et al. 2014) and Greece (Papadopoulos et al. 2018). Although ocular infection was detected in several species of wild animals (Otranto et al. 2009), only a few cases of infection were confirmed in wolves (Otranto et al. 2007, 2009; Mihalca et al. 2016).

In Serbia, thelaziosis was initially reported in dogs and cats (Gajić et al. 2014), with the first human case confirmed only a few years later (Tasić-Otašević et al. 2016). Despite the recent detection of eyeworm infection in foxes (Pavlović et al. 2017), data regarding wildlife from Serbia is still scanty. Therefore, the aim of this study was to investigate the occurrence and prevalence of zoonotic *T. callipaeda* in a population of gray wolf (*Canis lupus*) from different locations in Serbia.

Section Editor: Hiroshi Sato

✉ Bugarski-Stanojević Vanja  
vanjabs@ibiss.bg.ac.rs

<sup>1</sup> Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobođenja 18, Belgrade 11000, Serbia

<sup>2</sup> Institute for Biological Research “Siniša Stanković”, University of Belgrade, Despota Stefana Blvd.142, Belgrade 11060, Serbia

<sup>3</sup> Faculty of Biology, University of Belgrade, Studentski Trg 16, Belgrade 11000, Serbia

## Material and methods

During the hunting season, 2016/2017 and 2017/2018, 21 wolves (11 males and 10 females) of different ages (6 yearlings and 15 adults) were shot at 18 localities in Serbia. At necropsy, both conjunctival sacs of shot animals were visually examined and additionally flushed with saline solution (0.9% NaCl) in order to retrieve present eyeworms. Collected nematodes were stored in vials containing 70% ethanol. After parasite counting and morphological identification according to literature keys (Skrjabin et al. 1967; Otranto et al. 2003), DNA was extracted from one specimen per infected animal by DNeasy Blood and Tissue Kit (Qiagen). For PCR amplification of a 689-bp DNA fragment of the *cox1* gene, we used primers described in Casiraghi et al. (2001) and PCR conditions as in Otranto et al. (2005). Sequencing of the PCR products was obtained by a third party (Macrogen). Acquired sequences were analyzed using BioEdit ver. 7.2.5, FinchTV 1.4.0 chromatogram viewer (Geospiza Inc.) and compared with those currently available in GenBank by means of Basic Local Alignment Search Tool (BLAST) analysis.

## Results and discussion

Filiform, whitish-yellow nematodes were found in the conjunctival sacs of 38.1% (8/21) wolves (one yearling and seven adults) from seven localities with altitude ranging between 100 and 1100 m a.s.l. (Table 1; Fig. 1). In addition, both eyes were affected in all positive animals, with a total parasitic load ranging from four to 132 worms per animal. All collected nematodes were morphologically identified as *T. callipaeda* adult males (Fig. 2a, b) or adult females (Fig. 2c). Morphological findings were confirmed by molecular methods and exclusively haplotype 1 (h1) of *T. callipaeda* (GenBank accession no. AM042549; Otranto et al. 2005)



**Fig. 1** Adults of *T. callipaeda* in the conjunctival sac of a gray wolf (*Canis lupus*)

was detected in all analyzed samples ( $n = 8$ ). Additionally, representative sequences were deposited to the NCBI GenBank accession nos. MK034880, MK034881, MK034882, and MK034883.

Previous studies investigating thelaziosis in wolves in Europe were scarce and based on small sample size. Parasites have been reported in only six infected animals out of 18 examined, with the prevalence of infection ranging from 7.7 to 100%, depending on the study (Otranto et al. 2007, 2009; Mihalca et al. 2016). However, we showed the overall *T. callipaeda* prevalence of 38.1%, with a maximum parasitic load of 132 nematodes per animal. To the best of our knowledge, this is the first report of *T. callipaeda* in gray wolf from Serbia.

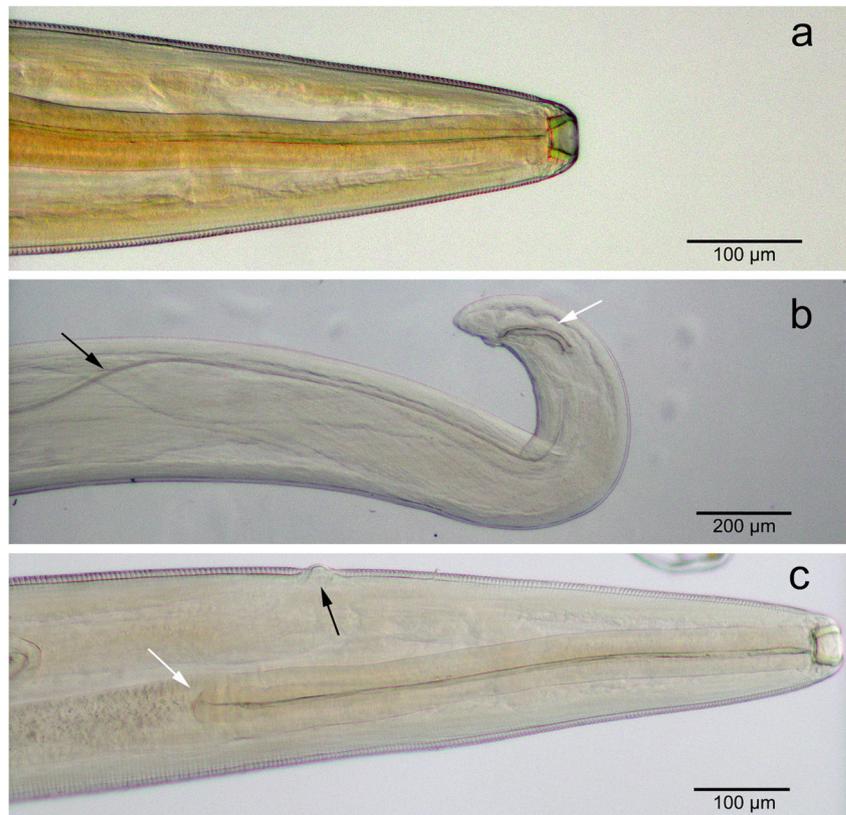
Wolves with the highest parasite burden detected in the present study (132 and 70 eyeworms, respectively) originated from the same Serbian county (Braničevo) as a pet dog from

**Table 1** Geographical distribution of *Thelazia callipaeda*-infected wolves in Serbia

Locality	Altitude (m)	Hunting date	Host data		Number of parasites		
			Age	Sex	Left/right eye	M/F	Total examined
Negotin	300	06 Jan 2017	Adult	Male	12/9	7/14	21
Sjenica	1100	14 Jan 2017	Adult	Male	7/5	2/10	12
Jagodina	400	15 Jan 2017	Adult	Male	4/3	1/6	7
Braničevo	100	28 Jan 2017	Adult	Female	44/88	27/105	132
Braničevo	100	28 Jan 2017	Adult	Male	28/42	16/54	70
Ražanj	300	11 Feb 2017	Adult	Female	14/21	9/26	35
Despotovac	550	15 Feb 2017	Yearling	Female	5/3	2/6	8
Svrljig	600	24 Dec 2017	Adult	Male	1/3	0/4	4

M, male; F, female

**Fig. 2** Light micrographs of *Thelazia callipaeda*. **a** Anterior end of *T. callipaeda* male. **b** Posterior end of *T. callipaeda* male with long (black arrow) and short (white arrow) spicules. **c** Anterior end of *T. callipaeda* female, showing vulva (black arrow) and esophago-intestinal junction (white arrow)



the previous study, harboring 91 nematodes (Gajić et al. 2014). Moreover, one out of three infected foxes originated from the same county (Pavlović et al. 2017). According to the high intensity of infection recorded in both domestic and wild animals, this study area might be considered as hyper-endemic for thelaziosis, although further investigations involving more animals and animal species should be conducted to confirm this hypothesis. Similar findings of simultaneous infections have also been reported in other host species such as dogs, cats, and foxes (summarized in Otranto et al. 2015).

All infected animals from the current study originated from localities with an altitude between 100 and 1100 m a.s.l. This is a broader altitude range than previously observed for canine and feline thelaziosis in Serbia (Gajić et al. 2014), but comparable to that in *T. callipaeda* hyper-endemic area in southern Italy, where different animal species were found infected (Otranto et al. 2009). In addition, bilateral eye infection detected in all positive wolves irrespective of an altitude of the locality suggests an intensive exposure of host animals and ubiquitous distribution of the fruitfly vector in Serbia, although there is no study investigating *P. variegata* so far.

Despite the limited number of examined animals ( $n = 21$ ), we showed that *T. callipaeda* has been widely present in wolves from Serbia. Importantly, only h1 haplotype was detected by the molecular assay, thus confirming the previous findings of a unique genetic haplotype of *T. callipaeda* circulating across Europe, regardless of the geographic region and

definitive host species (Otranto et al. 2005, 2007, 2009; Tasić-Otašević et al. 2016). The emergence of thelaziosis throughout Europe was rapid since the primary report in Italy, with complex roles of vectors, reservoir hosts, and the environment (Otranto and Deplazes 2019). However, the pan European distribution of the vector *Phortica variegata* is crucial to explain the spread of *T. callipaeda* (Máca and Otranto 2014). The authors further explain that wild canids and felids are exposed to the vector since they frequently share the same environment-forested, meadow hilly areas, regions around rivers with high relative humidity, which is essential for the spread of *T. callipaeda*. The importance of wild canids is confirmed by the high prevalence of infection, with foxes being the most reported wildlife host in Europe (Otranto et al. 2009; Hodžić et al. 2014; Čabanová et al. 2018). Nevertheless, the high prevalence of thelaziosis observed in our research (38.1%) along with the wolves' tendency to move a long distance (more than 1000 km; see Ražen et al. 2016) emphasize the role of gray wolf as a natural reservoir for *T. callipaeda* in its sylvatic life cycle as well as the role in the dissemination of infection within its natural territories. Results of genetic studies on populations of wolves from the Balkan area (Moura et al. 2014; Karamanlidis et al. 2016) suggest that wolves stroll and breed among bordering countries and may support the introduction of pathogens such as *T. callipaeda*, further in range. Our study, therefore, corroborates the competence of gray wolf as a definitive host for *T. callipaeda*

(Otranto et al. 2007, 2009) as well as seasonality of parasite's reproductive cycle (Otranto et al. 2004), since only adult parasites were found in infected wolves shot between December and February.

In order to complete epidemiological puzzle regarding this emerging zoonosis, the role of more abundant wild carnivore species (i.e., foxes and jackals) should be investigated in future studies. Better awareness of this parasitosis is vital to limit the risk of further infections in both, humans and animals, in European countries.

**Acknowledgments** We are most grateful to Srđan Vučković and Marija Spasić who kindly helped in sample collection.

**Funding information** This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant nos. 173006 and 173025).

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

### References

- Anderson RC (2000) Nematode parasites of vertebrates: their development and transmission, 2nd edn. CABI Publishing, Wallingford, p 672
- Čabanová V, Miterpáková M, Oravec M, Hurníková Z, Jerg S, Nemčíková G, Červenská MB (2018) Nematode *Thelazia callipaeda* is spreading across Europe. The first survey of red foxes from Slovakia. Acta Parasitol 63(1):160–166. <https://doi.org/10.1515/ap-2018-0018>
- Casiraghi M, Anderson TJC, Bandi C, Bazzocchi C, Genchi C (2001) A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology 122(1):93–103. <https://doi.org/10.1017/S0031182000007149>
- Colella V, Kirkova Z, Fok É, Mihalca AD, Tasić-Otašević S, Hodžić A, Dantas-Torres F, Otranto D (2016) Increase in eyeworm infections in eastern Europe. Emerg Infect Dis 22(8):1513–1515. <https://doi.org/10.3201/eid2208.160792>
- Dorchies P, Chaudieu G, Siméon LA, Cazalot G, Cantacessi C, Otranto D (2007) First reports of autochthonous eyeworm infection by *Thelazia callipaeda* (Spirurida, Thelaziidae) in dogs and cat from France. Vet Parasitol 149(3–4):294–297. <https://doi.org/10.1016/j.vetpar.2007.08.005>
- Gajić B, Bogunović D, Stevanović J, Kulišić Z, Simeunović P, Stanimirović Z (2014) Canine and feline thelaziosis caused by *Thelazia callipaeda* in Serbia. Acta Vet-Beograd 64(4):447–455. <https://doi.org/10.2478/acve-2014-0042>
- Hodžić A, Latrofa MS, Annoscia G, Alić A, Beck R, Lia RP, Dantas-Torres F, Otranto D (2014) The spread of zoonotic *Thelazia callipaeda* in the Balkan area. Parasit Vectors 7(1):352. <https://doi.org/10.1186/1756-3305-7-352>
- Karamanlidis AA, Gabriel Hernando M, Georgiadis L, Kusak J (2016) Activity, movement, home range and habitat use of an adult gray wolf in a Mediterranean landscape of northern Greece. Mammalia. <https://doi.org/10.1515/mammalia-2015-0091>
- Máca J, Otranto D (2014) Drosophilidae feeding on animals and the inherent mystery of their parasitism. Parasite Vector 7:516. <https://doi.org/10.1186/s13071-014-0516-4>
- Magnis J, Naucke TJ, Mathis A, Deplazes P, Schnyder M (2010) Local transmission of the eye worm *Thelazia callipaeda* in southern Germany. Parasitol Res 106:715–717. <https://doi.org/10.1007/s00436-009-1678-4>
- Malacrida F, Hegglin D, Bacciarini L, Otranto D, Nägeli F, Nägeli C, Bernasconi C, Scheu U, Balli A, Marengo M, Togni L, Deplazes P, Schnyder M (2008) Emergence of canine ocular thelaziosis caused by *Thelazia callipaeda* in southern Switzerland. Vet Parasitol 157: 321–327. <https://doi.org/10.1016/j.vetpar.2008.07.029>
- Mihalca AD, Ionică AM, D'Amico G, Daskalaki AA, Deak G, Matei IA, Şimonca V, Iordache D, Modrý D, Gherman CM (2016) *Thelazia callipaeda* in wild carnivores from Romania: new host and geographical records. Parasite Vector 9(1):350. <https://doi.org/10.1186/s13071-016-1628-9>
- Miró G, Montoya A, Hernández L, Dado D, Vázquez MV, Benito M, Villagrasa M, Brianti E, Otranto D (2011) *Thelazia callipaeda*: infection in dogs: a new parasite for Spain. Parasite Vector 4:148. <https://doi.org/10.1186/1756-3305-4-148>
- Moura AE, Tsingarska E, Dąbrowski MJ, Czarnomska SD, Jędrzejewska B, Pilot M (2014) Unregulated hunting and genetic recovery from a severe population decline: the cautionary case of Bulgarian wolves. Conserv Genet 15:405–417. <https://doi.org/10.1007/s10592-013-0547-y>
- Otranto D, Deplazes P (2019) Zoonotic nematodes of wild carnivores. Int J Parasitol Parasites Wildl 9:370–383. <https://doi.org/10.1016/j.ijppaw.2018.12.011>
- Otranto D, Lia RP, Traversa D, Giannetto S (2003) *Thelazia callipaeda* (Spirurida, Thelaziidae) of carnivores and humans: morphological study by light and scanning electron microscopy. Parasitologia 45: 125–133
- Otranto D, Lia RP, Buono V, Traversa D, Giangaspero A (2004) Biology of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts. Parasitology 129(5):627–633
- Otranto D, Testini G, De Luca F, Hu M, Shamsi S, Gasser RB (2005) Analysis of genetic variability within *Thelazia callipaeda* (Nematoda: Thelazioidea) from Europe and Asia by sequencing and mutation scanning of mitochondrial cytochrome *c* oxidase subunit 1 gene. Mol Cell Probes 19:306–313. <https://doi.org/10.1016/j.mcp.2005.05.001>
- Otranto D, Cantacessi C, Testini G, Lia RP (2006) *Phortica variegata* as an intermediate host of *Thelazia callipaeda* under natural conditions: evidence for pathogen transmission by a male arthropod vector. Int J Parasitol 36(10–11):1167–1173. <https://doi.org/10.1016/j.ijpara.2006.06.006>
- Otranto D, Cantacessi C, Mallia E, Lia RP (2007) First report of *Thelazia callipaeda* (Spirurida, Thelaziidae) in wolves in Italy. J Wildl Dis 43(3):508–511. <https://doi.org/10.7589/0090-3558-43.3.508>
- Otranto D, Dantas-Torres F, Mallia E, DiGeronimo PM, Brianti E, Testini G, Traversa D, Lia RP (2009) *Thelazia callipaeda* (Spirurida, Thelaziidae) in wild animals: report of new host species and ecological implications. Vet Parasitol 166(3–4):262–267. <https://doi.org/10.1016/j.vetpar.2009.08.027>
- Otranto D, Cantacessi C, Dantas-Torres F, Brianti E, Pfeffer M, Genchi C, Guberti V, Capelli G, Deplazes P (2015) The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part II: Helminths and arthropods. Vet Parasitol 213(1–2):24–37. <https://doi.org/10.1016/j.vetpar.2015.04.020>
- Papadopoulos E, Komnenou A, Thomas A, Ioannidou E, Colella V, Otranto D (2018) Spreading of *Thelazia callipaeda* in Greece. Transbound Emerg Dis 65(1):248–252. <https://doi.org/10.1111/tbed.12626>
- Pavlović I, Jakić-Dimić D, Kureljušić B, Čirović D, Jezdimirović N, Drobnjak M (2017) First occurrence of *Thelazia callipaeda* in foxes

- (*Vulpes vulpes* L.) in Serbia. *Balk J Wildlife Res* 4(1):1–5. <https://doi.org/10.15679/bjwr.v4i1.31>
- Ražen N, Brugnoli A, Castagna C, Groff C, Kaczensky P, Kljun F, Knauer F, Kos I, Krofel M, Luštrik R, Majić A, Rauer G, Righetti D, Potočnik H (2016) Long-distance dispersal connects Dinaric-Balkan and Alpine grey wolf (*Canis lupus*) populations. *Eur J Wildl Res* 62(1):137–142. <https://doi.org/10.1007/s10344-015-0971-z>
- Rossi L, Bertaglia P (1989) Presence of *Thelazia callipaeda* Railliet and Henry, 1910, in Piedmont, Italy. *Parassitologia* 31:167–172
- Skrjabin KI, Sobolev AA, Ivashkin VM (1967) Spirurata of animals and man and the disease caused by them. Part 4: Thelazioidea. In: Skrjabin KI (ed) *Essential of nematology*, vol XVI. Academy of Sciences of the USSR, Moscow, p 624 in Russian
- Tasić-Otašević S, Gabrielli S, Trenkić-Božinović M, Petrović A, Gajić B, Colella V, Momčilović S, Cancrini G, Otranto D (2016) Eyeworm infections in dogs and in a human patient in Serbia: a one health approach is needed. *Comp Immunol Microbiol Infect Dis* 45:20–22. <https://doi.org/10.1016/j.cimid.2016.01.003>
- Vieira L, Rodrigues TF, Costa A, Diz-Lopes D, Machado J, Coutinho T, Tuna J, Latrofa MS, Cardoso L, Otranto D (2012) First report of canine ocular thelaziosis by *Thelazia callipaeda* in Portugal. *Parasit Vectors* 5:124. <https://doi.org/10.1186/1756-3305-5-124>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.