Suspected autochthonous *Thelazia callipaeda* infection in a dog in northern Germany

Sophia L. Lebedewa 1 · Kevin Tkocz 2 · Peter-Henning Clausen 1 · Ard M. Nijhof 1

Received: 22 January 2020 / Accepted: 5 October 2020 / Published online: 13 October 2020 © The Author(s) 2020

**Abstract**

A 12-year old Elo dog was presented with recurring symptoms of conjunctivitis in November 2019. A single whitish nematode was found upon inspection of the eye and identified as a *Thelazia callipaeda* male. The morphological identification of the eye worm was supported by analysis of a partial cytochrome c oxidase I (cox1) gene sequence. The dog lived in Lower Saxony, northwestern Germany, and had not visited regions known to be endemic for *T. callipaeda*. This suggests that a local transmission cycle of this zoonotic nematode may exist in Germany.

**Keywords** *Thelazia callipaeda* · Germany · Cytochrome c oxidase I · Zoonosis

**Introduction**

*Thelazia callipaeda* (Spirurida, Thelaziidae) are nematodes that occur in the eyes of carnivores, particularly dogs and foxes, as well as lagomorphs and sometimes humans (Gama et al. 2016; Otranto and Deplazes 2019). The parasite was first described from Asia in 1910 (Railliet and Henry 1910), where it is widespread. The first case of canine thelaziosis in Europe was described in Italy in the late 1980s (Rossi and Bertaglia 1989), and the distribution of this ‘oriental eye worm’ has since extended to larger parts of Europe, with enzootic areas in southern Europe (do Vale et al. 2019).

Adult *T. callipaeda* lives in the orbital cavity of the definitive host, where they reach sexual maturity after about 3–4 weeks post-infection and can survive for more than 1 year (Faust 1928; Kozlov 1962; Anderson 2000; Otranto et al. 2004). First-stage larvae (L1) produced by the larviparous female eye worm are released in the lachrymal secretions. The non-biting fruit fly *Phortica variegata* that feed on these secretions can become infected with the L1 and act as intermediate hosts and vector for this nematode in Europe (Otranto and Dantas-Torres 2015). Under natural conditions, male *P. variegata* flies appear to be the main vectors for *T. callipaeda*, as female *P. variegata* flies prefer fruit as their food source, whereas males show a more zoophilic behaviour (Otranto et al. 2006). In male *P. variegata*, the L1 migrates from the crop to the testes where it develops to the infective L3 stage. The L3 migrates back to the proboscis from where it is transmitted to the definitive host when the fly feeds on ocular secretions again (Otranto et al. 2005). Further development to the adult stage takes place on the surface of the eye.

Infections with *T. callipaeda* may be subclinical but can also cause symptoms of conjunctivitis such as lacrimation and pruritus and, in severe cases, keratitis and ulceration (Marino et al. 2020).

Canine thelaziosis caused by *T. callipaeda* has previously been reported from Germany and was associated with travel to an endemic region in Italy (Hermosilla et al. 2004) or originated from a region close to the French border in southern Germany (Magnis et al. 2010). We here report on a case of canine thelaziosis in a dog from northwestern Germany who had not entered regions known to be endemic for *T. callipaeda*, making a local transmission likely.

**Material and methods**

**Animal**

A 12-year-old, female Elo dog living in Lower Saxony, northwestern Germany, was presented to a veterinarian in
November 2019 for a check-up for distichiasis since it showed recurring unilateral symptoms of a slight conjunctivitis and pruritus. The symptoms had first appeared early August 2019, and the owner initially treated it with homoeopathic eye drops, which provided only short-term relief. During the clinical examination, a single whitish worm was detected in the left eye. The dog was subsequently treated with a spot-on product containing moxidectin and imidacloprid (Prinovox®, Virbac). Clinical symptoms were not observed during clinical examinations conducted at 5 days and 2 months post-treatment. The dog had been treated in July 2019 with a spot-on product containing permethrin and imidacloprid (Advantix®, Bayer Animal Health) for tick bite prevention.

The dog had crossed the German-French border near Saarbrücken once for a single day in April 2017 and had travelled to Denmark and Sweden in August 2019, after the conjunctivitis had appeared for the first time.

**Morphological identification**

The adult nematode was initially stored in Ringer’s solution and submitted to the Institute for Parasitology and Tropical Veterinary Medicine, where it was stored in 70% ethanol before examination using a Stemi 2000-C microscope (Carl Zeiss, Jena, Germany).

**Molecular identification**

A ~ 700-bp fragment of the cytochrome c oxidase I (cox1) gene was amplified using primers COI_Nema_fw (5′-GAAGTTCTAATCATAARGATATTGG-3′) and COI_Nema_Rv (5′-ACCTCAGGATGACCAAAAAA YCAA-3′) (Duscher et al. 2015). PCR reactions (20 μl) were performed with 0.2 mM dNTPs, 250 nM of each primer, 0.1 U Phusion Hot Start II High-Fidelity DNA polymerase (Thermo Scientific) and 2 μl template DNA in 1× HF buffer under the following cycling conditions: an initial denaturation step at 98 °C for 30 s, followed by 40 cycles of 98 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s and a final elongation step at 72 °C for 5 min. The amplicons were subsequently purified using DNA Clean and Concentrator kit (Zymo Research), cloned using the StrataClone Blunt PCR Cloning Kit (Agilent) using the manufacturer’s instructions. Plasmids containing an insert of the expected size were sequenced in both directions (LGC Genomics, Berlin, Germany). The resulting sequence was edited using BioEdit (v7.2.5).

**Results**

The nematode was morphologically identified as a male *Thelazia callipaeda* worm (Fig. 1).

This morphological identification was confirmed by amplification and subsequent sequencing of a partial fragment of the *cox1* gene sequence. This sequence was 100% (683/683 nt) identical to the *cox1* sequence of a complete mitochondrial genome of *T. callipaeda* (GenBank Accession Number AP017700) that was published as part of the 50 Helminth Genomes initiative (https://www.sanger.ac.uk/science/collaboration/50HGP) and 99.8% (498/499 nt) identical to haplotype h1 (GenBank Accession Number AM042549), the single haplotype circulating in Europe (Otranto et al. 2005). The single nucleotide difference with AM042549 was located in the sequence of the forward primer used in that study.

**Discussion**

Since its first detection in Italy in the 1980s, *T. callipaeda* has spread across Europe, with recent cases of autochthonous thelaziosis in carnivores from Bulgaria (Colella et al. 2016), Slovakia (Cabanova et al. 2017), Hungary (Farkas et al. 2018), Moldova (Dumitrache et al. 2019) and Austria (Hodzic et al. 2019), as well as several reported human cases (Paradzik et al. 2016; Tasic-Otasevic et al. 2016; Dolff et al. 2020). Important factors in the expansion of canine thelaziosis include the travelling of infected dogs to/from endemic regions and the presence of a sylvatic cycle mainly maintained by foxes (Otranto et al. 2009; do Vale et al. 2019).

Previous reports from Germany concerned a dog that had recently visited Italy (Hermosilla et al. 2004), two dogs and a cat with a recent presence in endemic countries (Silva et al. 2020) and a probable autochthonous case of a dog in...
southwestern Germany, near the French border (Magnis et al. 2010). An interesting aspect of the case presented here is the fact that the dog lived in northern Germany and had not entered regions known to be endemic for T. callipaeda. Her southernmost visit had been a daytrip to the German-French border area near Saarbrücken in April 2017. Although this region is not known to be endemic for T. callipaeda, it cannot be completely excluded that the initial infection occurred here, as the longevity of adult nematodes was reported to be more than 1 year (Faust 1928). However, the fact that the conjunctivitis symptoms occurred early August 2019 speaks more in favour of a local transmission in northern Germany. Phorcta variegata, the intermediate host of T. callipaeda, is present in Germany (Bächli 2019), and a recent ecological niche model showed that large areas of Europe, including Lower Saxonony where the dog lived, are suitable for the development of P. variegata (Palfreyman et al. 2018). The lachryphagous activity of male P. variegata was shown to be associated with temperature, with 7 to 11 °C being required as a minimum temperature for the start of activity (Pombi et al. 2020). In Lower Saxony, daily average temperatures above 7 °C were reported from May to October 2019 (wetterkontor.de 2020), suggesting that this will not form a limitation for the transmission of T. callipaeda. However, more research, for instance, into the local activity of P. variegata and the prevalence of T. callipaeda in domestic and wild carnivores in Germany would be required to confirm the presence of a local transmission cycle.

This case report shows that veterinarians in northern Europe should be aware of canine thelaziosis, as it presents an emerging vector-borne disease with zoonotic implications in Europe.

Acknowledgements We would like to thank the animal’s owner for her cooperation in this study.

Funding Open Access funding enabled and organized by Projekt DEAL. AMN received financial support by the Federal Ministry of Education and Research (BMBF) under project number 01KI1720 as part of the ‘Research Network Zoonotic Infectious Diseases’.

Compliance with ethical standards

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

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Anderson RC (2000) Order Spirurida - suborder Spirurina nematode parasites of vertebrates their development and transmission, 2nd edn. CABI Publishing, Oxon, pp 383–590

Bächli G (2019) TaxoDros, the database on taxonomy of Drosophilidae. Cabanova V, Kocak P, Vichova B, Miterpavokova M (2017) First autochthonous cases of canine thelaziosis in Slovakia: a new affected area in Central Europe. Parasit Vectors 10(1):179

Colella V, Kirkova Z, Fok E, Mihalea AD, Tasic-Otasevic S, Hodzic A, Dantas-Torres F, Otranto D (2016) Increase in eyeworm infections in Eastern Europe. Emerg Infect Dis 22(8):1513–1515

do Vale B, Lopes AP, da Fonseca Fontes M, Silvestre M, Cardoso L, Coelho AC (2019) Thelaziosis due to Thelazia callipaeda in Europe in the 21st century-a review. Vet Parasitol 275:108957

Dolff S, Kehrmann J, Eisermann P, Dalbah S, Tappe D, Rating P (2020) Case report: Thelazia callipaeda eye infection: the first human case in Germany. Am J Trop Med Hyg 102(2):330–351

Dumitrache MO, Ionica AM, Voimitchi E, Chavdar N, D’Amico G (2019) First report of canine ocular thelaziosis in the Republic of Moldova. Parasit Vectors 12(1):505

Duscher GG, Harl J, Fuehrer HP (2015) Evidence of Troglocrema acutum and Skrjabingylus sp. coinfection in a polecat from Lower Austria. Helminthologia 52(1):63–66

Farkas R, Takaes N, Gyurkovszky M, Henszelnmann N, Kiesergely J, Balka G, Solymosi N, Vass A (2018) The first feline and new canine cases of Thelazia callipaeda (Spirurida: Thelazidae) infection in Hungary. Parasit Vectors 11(1):338

Faust EC (1928) Studies on Thelazia callipaeda Railliet and Henry, 1910. J Parasitol 15(2):75

Gama A, Pires I, Canado M, Coutinho T, Lopes AP, Latrofa MS, Cardoso L, Dantas-Torres F, Otranto D (2016) First report of Thelazia callipaeda infection in wild European rabbits (Oryctolagus cuniculus) in Portugal. Parasit Vectors 9(1):236

Hermosilla C, Herrmann B, Bauer C (2004) First case of Thelazia callipaeda infection in a dog in Germany. Vet Rec 154(18):568–569

Hodzic A, Payer A, Duscher GG (2019) The first autochthonous case of feline ocular thelaziosis in Austria. Parasitol Res 118(4):1321–1324

Kozlov DP (1962) The life cycle of nematode Thelazia callipaeda. Doklady Akademy Nauk SSSR 142:732–733

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(3):715–717

Marino V, Galvez R, Montoya A, Mascarén A, Hernandez M, Barrera JP, Dominguez I, Zenker C, Checa R, Sarquis J, Miro G (2020) Spain as a dispersion model for Thelazia callipaeda eyeworm in dogs in Europe. Prev Vet Med 175:104883

Otranto D, Cantacessi C, Testini G, Lia RP (2006) Phorcta variegata as an intermediate host of Thelazia callipaeda under natural conditions: evidence for pathogen transmission by a male arthropod vector. J Int Parasitol 36(10–11):1167–1173

Otranto D, Dantas-Torres F (2015) Transmission of the eyeworm Thelazia callipaeda: between fantasy and reality. Parasit Vectors 8(1):273

Otranto D, Dantas-Torres F, Mallia E, DiGeronimo PM, Brianti E, Testini G, Traversa D, Lia RP (2009) Thelazia callipaeda (Spirurida, Thelazidae) in wild animals: report of new host species and ecological implications. Vet Parasitol 166(3–4):262–267
Otranto D, Deplazes P (2019) Zoonotic nematodes of wild carnivores. Int J Parasitol Parasites Wildl 9:370–383
Otranto D, Lia RP, Buono V, Traversa D, Giangaspero A (2004) Biology of Thelazia callipaeda (Spirurida, Thelaziidae) eye worms in naturally infected definitive hosts. Parasitology 129(Pt 5):627–633
Otranto D, Lia RP, Testini G, Troccoli A, Shen JL, Wang ZX (2005) Nematode biology and larval development of Thelazia callipaeda (Spirurida, Thelaziidae) in the drosophilid intermediate host in Europe and China. Parasitology 131(Pt 6):847–855
Otranto D, Testini G, De Luca F, Hu M, Shamsi S, Gasser RB (2005) Analysis of genetic variability within Thelazia callipaeda (Nematoda: Thelazioida) from Europe and Asia by sequencing and mutation scanning of the mitochondrial cytochrome c oxidase subunit 1 gene. Mol Cell Probes 19(5):306–313
Palfreyman J, Graham-Brown J, Caminade C, Gilmore P, Otranto D, Williams DJL (2018) Predicting the distribution of Phortica variegata and potential for Thelazia callipaeda transmission in Europe and the United Kingdom. Parasit Vectors 11(1):272
Paradzik MT, Samardzic K, Zivcencjak T, Martinkovic F, Janjetovic Z, Miletic-Medved M (2016) Thelazia callipaeda—first human case of thelaziosis in Croatia. Wien Klin Wochenschr 128(5–6):221–223
Pombi M, Marino V, Jaenike J, Graham-Brown J, Bernardini I, Lia RP, Beugnet F, Miro G, Otranto D (2020) Temperature is a common climatic descriptor of lachryphagous activity period in Phortica variegata (Diptera: Drosophilidae) from multiple geographical locations. Parasit Vectors 13(1):89
Railliet A, Henry A (1910) Nouvelles observations sur les Thelazies, nematodes parasites de l’œil. Compt Rend Soc Biol 68:783–778
Rossi L, Bertaglia PP (1989) Presence of Thelazia callipaeda Railliet & Henry, 1910, in Piedmont, Italy. Parasitologia 31(2–3):167–172
Silva LMR, Spoerel S, Wiesner L, Klein M, Pantchev N, Taubert A, Hermosilla C (2020) Ophthalmic Thelazia callipaeda infections: first feline and new canine imported cases in Germany. Parasitol Res 119(9):3099–3104
Tasic-Otasevic S, Gabrielli S, Trenkic-Bozinovic M, Petrovic A, Gajic B, Colella V, Momcilovic 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

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