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Feline thelaziosis caused by *Thelazia callipaeda* in Portugal

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Abstract

Thelazia callipaeda (Spirurida, Thelaziidae) is a nematode that lives in the conjunctival sac of domestic and wild carnivores, rabbits and humans. causing mild to severe symptoms (e.g., conjunctivitis, lacrimation, epiphora, blepharospasm, keratitis and even corneal ulceration) in infected animals. This report describes an autochthonous case of thelaziosis in a cat from the central region of Portugal, representing the most occidental record of thelaziosis in Europe. Adult nematodes recovered from alive animal were morphological identified as *T. callipaeda*. A portion of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox* 1) from nematode specimens was amplified by PCR. *Cox*1 sequences of all specimens were identical to *T. callipaeda* haplotype 1. Additionally to these findings, a recent description of thelaziosis in the northern region of Portugal suggest that *T. callipaeda* has successfully established in Portugal.

1. Introduction

Thelaziosis is caused by 16 species of nematodes belonging to the genus *Thelazia* (Spirurida, Thelaziidae). *Thelazia callipaeda* (Railliet and Henry, 1910) and *Thelazia californiensis* (Price, 1930) are the only parasites with zoonotic concern (Anderson, 2000; Otranto and Eberhard, 2011). *T. callipaeda* infects canids (*Canis familiaris*, *Nyctereutes procyonoides*, *Vulpes fulva*), cats (*Felis catus*), rabbits (*Oryctolagus cuniculus*) and, rarely humans. Adult parasites live in the conjunctival sac of the definitive hosts, affecting the conjunctiva and the anterior chamber of the eye (Otranto and Traversa, 2005).

In Europe, thelaziosis caused by *T. callipaeda* is transmitted by *Phortica variegata* (Diptera, Drosophilidae), a small secretophagous fly, usually known as “fruit fly” . This

vector feeds on fruits, vegetables and also on lachrymal secretions of domestic animals and wildlife (Otranto et al., 2005a; Otranto et al., 2006a). *T. callipaeda* adult female releases first stage larvae which are ingested by *P. variegata* when feeding on lachrymal secretions of infected animals (Otranto and Traversa, 2005). In the vector, larvae moult twice and the third stage larvae migrate to the labella. The third stage larvae are transmitted to a receptive host while flies feed on the eyes surface of the animals (Otranto et al., 2005a; Otranto et al., 2006a). It has been suggested that *P. variegata* males present a zoophilic behavior (while females feed on fruit and other vegetable matters) which might be related to environmental and biological factors, as well as to dietary needs (e.g., high-protein supplementation) of males (Otranto et al., 2006a; Otranto et al., 2006b).

Thelaziosis was formerly known as the “oriental eyeworm”, since its distribution was initially confined to Asian countries (i.e., Indonesia, China, Thailand, Korea, India and Japan), but the parasite has expanded gradually throughout Europe, with hyper endemic foci in Italy (Otranto et al., 2003a), being identified in wild animals, such as foxes and wolves (Otranto et al., 2007; Otranto et al., 2009). Isolated cases in dogs of *T. callipaeda* were initially reported in France (Chermette et al., 2004) and Germany (Hermosilla et al., 2004).These dogs had been travelling to northern Italy during the summer season. Additionally, autochthonous cases of thelaziosis in dogs and cats from France (Dorchies et al., 2007), Switzerland (Malacrida et al., 2008) and Germany (Magnis et al., 2010) have been described in recent years. Furthermore, the recent findings of *T. callipaeda* in dogs and cats in Spain (Miró et al., 2011) and in the northern of Portugal (Rodrigues et al., 2012; Vieira et al., 2012) suggest a progressive dissemination of the parasite towards western European countries.

An increased number of infections is usually reported in spring and summer, when the vector is active. Adult parasites remain viable for more than one year, explaining the dynamics pattern of the parasitism by the occurrence of two peaks of infection; one in the early summer (adult parasites that overwinter) and other in late summer (adults developing from infectious stages laid by the vector in early summer) (Otranto et al., 2004; Otranto and Traversa, 2005). Infected animals may present ocular abnormalities (e.g., lacrimation, ocular congestion, exudative conjunctivitis, photosensitivity, epiphora, blepharospasm and, even, keratitis or corneal ulceration), possibly associated to the transversal striation of the parasite cuticle (Otranto and Traversa, 2005).

This paper describes a case of *T. callipaeda* infection in a cat from the central region of Portugal without a record of previous travelling to endemic regions.

2. Material and methods

2.1 Case report

A four-year old common European female cat weighing 4 kg showed ocular discharge of the right eye and displayed abnormal grooming behavior. The animal lived in a small village at the District of Coimbra in the center of Portugal (40,1ºN and 8.5ºW).

At the physical examination the animal was in good body condition, with a pronounced blepharospasm, photophobia, purulent secretions and mild conjunctival edema on the right eye. No other abnormalities were detected on physical examination. Following administration of an ocular anesthetic (oxibupocraine hydrochloride, Anestocil®) the ophthalmic examination revealed the absence of corneal lesions (fluorescein test negative), and the presence of adult nematodes in the lachrymal sac.

Mechanical removal of worms was performed by washing the eye with sterile physiologic saline solution (NaCl 0.9%) recovering a total of sixteen worms. The animal was treated with 1% doramectin solution (Dectomax®, off-label use) subcutaneously (100 mcg/kg of body weight), repeated 2 weeks later, as well as topic application of 1% fucsidic acid (Fucithalmic®) for 7 days (twice a day). The animal recovered and no relapses occurred after treatment (follow-up time of 6 months).

Worms collected from the animal eye were stored in 70% ethanol and sent to the Parasitological Unit of the Department of Veterinary Medicine (University of Bari, Italy) for morphological and molecular identification.

2.2 Morphological and molecular identification

Nematodes were morphologically identified according to the keys of Skrjabin et al. (1967) and Otranto et al. (2003b). Briefly, specimens were identified as *T. Callipaeda* based on the presence of five pairs of large post-cloacal papillae in the ventral position in the males and position of the vulva anterior to the oesophago-intestinal junction in the females. Eight nematodes (one male and seven females) were molecularly characterized as previously described by Otranto et al. (2005b), in order to confirm the morphological identification. Molecular identification by PCR was performed amplificating a portion of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox*1, 689 bp). The amplicons were then sequenced and the sequences aligned, using the ClustalX. The alignments were compared with those in public databases (i.e., NCBI at http://www.ncbi.nlm.nih.gov/).

3. Results and discussion

From the sixteen worms morphologically identified, one male and seven females were molecularly identified as *T. callipaeda.* Morphologically, female worms presented the vulva anterior to the oesophago-intestinal junction (Figure 1); and males had large post-cloacal papillae (Figure 2) as well as short and long spicules at the posterior end (Figure 3). All *cox*1 sequences of the specimens were identical to the sequence of *T. callipaeda* haplotype 1 (GenBank accession number AM042549).

The infected animal was an indoor cat with free access to the backyard of a house, a farm with fruit trees (i.e., apples, oranges and kiwis) and vegetables (i.e. strawberries, kale, tomatoes).

Since the infected cat had never been travelling outside the region or abroad, the transmission of *T. callipaeda* was most likely to be autochthonous. The region (40,1ºN and 8,5ºW) located at the center of Portugal, is about 64 m of altitude on the Atlantic Ocean costal area, being characterized by a Mediterranean climate similar to those described in other European cases (Otranto et al., 2003a; Dorchies et al., 2007; Miró et al., 2011).

The findings here reported strongly suggest that *T. callipaeda* is present in the region. It is well known that *P. variegata* is the only vector of *T. callipaeda* in Europe, both under laboratory and natural conditions (Otranto et al., 2005a; Otranto et al., 2006b). Moreover as Otranto et al. (2006c) pointed out vast areas of Europe are suitable for the development of *P. variegata*, indicating the spreading of the infection by *T. callipaeda* to areas previously considered as non-endemic. Furthermore, reinforce that this worm has found suitable conditions to complete its life cycle in this region.

Haplotype 1 herein identified is identical to the one found in the northern region of Portugal (Rodrigues et al., 2012; Vieira et al., 2012) and Spain (Miró et al., 2011) as well as in other European cases (Otranto et al., 2009). From the above described, the parasite in this case study was possibly introduced in the area by animals travelling throughout regions where thelaziosis is endemic (e.g. Spain, France, Italy, and Switzerland) or by wildlife carnivores acting as reservoirs of infection which circulate through border regions (Otranto et al., 2007).

Primary treatment for thelaziosis includes mechanical removal of worms. Additionally, it is recommended to treat infected animals with a macrocyclic lactone (e.g., moxidectin, milbemycin oxime), since the total mechanical removal of worms is not ensured (Bianciardi and Otranto, 2005; Ferroglio et al., 2008). In the present report, the mechanical removal of adult worms from the cat's eye was followed by a 1% injectable formulation of doramectin (Dectomax®, off-label use) administration. No post-treatment relapses were reported (follow-up time of 6 months) neither adverse reactions related to the off-label use of doramectin.

These findings, together with previous reports of thelaziosis in the northern of Portugal, highlight the need to conduct further studies assessing the real distribution of this parasite among domestic and wild carnivores. Furthermore the zoonotic potential of this parasite should also not be neglected.

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168 **References**

169 Anderson, R.C., 2000. Nematode parasites of vertebrates: Their development and
170 transmission. 2nd edition. CABI Publishing, Guilford, UK, pp 404-407.

171 Bianciardi, P., Otranto, D., 2005. Treatment of dog thelaziosis caused by Thelazia
172 callipaeda (Spirurida, Thelaziidae) using a topical formulation of imidacloprid 10% and
173 moxidectin 2.5%. *Vet. Parasitol*. 129(1-2), 89-93.

174 Chermette, R., Guillot, J., Bussièras, J., 2004. Canine ocular thelaziosis in Europe. *Vet.*
175 *Rec.* 154, 248.

176 Dorchies, P., Chaudieu, G., Siméon, L., Cazalot, G., Cantacessi, C., Otranto D., 2007.
177 First report of autochthonous eye infection by *Thelazia callipaeda* in dogs and cats from
178 France. *Vet. Parasitol*. 149, 294-297.

179 Ferroglio, E., Rossi, L., Tomio, E., Schenker, R., Bianciardi, P., 2008. Therapeutic and
180 prophylactic efficacy of milbemycin oxime (Interceptor) against *Thelazia callipaeda* in
181 naturally exposed dogs. *Vet. Parasitol*. 154(3-4), 351-353.

182 Hermosilla, C., Bauer, C., Herrmann, B., 2004. First case of *Thelazia callipaeda*
183 infection in a dog in Germany. *Vet. Rec*. 154, 568-569.

184 Magnis, J., Naucke, T.J., Mathis, A., Deplazes, P., Schnyder, M., 2010. Local
185 transmission of the eye worm *Thelazia callipaeda in* southern Germany. *Parasitol. Res*.
186 106(3), 715-717.

187 Malacrida, F., Hegglin, D., Bacciarini, L., Otranto, D., Nägeli, F., Nägeli, C.,
188 Bernasconi, C., Scheu, U., Balli, A., Marenco, M., Togni, L., Deplazes, P., Schnyder,
189 M., 2008. Emergence of canine ocular thelaziosis caused by *Thelazia callipaeda* in
190 southern Switzerland. *Vet. Parasitol*. 157 (3-4), 321-327.

191 Miró, G., Montoya, A., Hernández, L., Dado, D., Vázquez, M. M., Benito, M.,
192 Villagrassa, M., Brianti, E., Otranto, D., 2011. *Thelazia callipaeda*: infection in dogs, a
193 new parasite for Spain. *Parasit Vectors*, 4,148.
194 Otranto, D., Brianti, E., Cantacessi, C., Lia, R.P., Máca, J., 2006a. The zoophilic fruitfly
195 *Phortica variegata*: morphology, ecology and biological niche. *Med. Vet. Entomol*. 20,
196 358–364.
197 Otranto, D., Brianti, E., Cantacessi, C., Lia, R.P., Máca, J., 2006c. Morphology, ecology
198 and biological niches of the zoophilic fruitfly, *Phortica variegata*. *Med. Vet. Entomol*.
199 20, 358- 364
200 Otranto, D., Cantacessi, C., Mallia, E., Lia, R.P., 2007. First report of *Thelazia*
201 *callipaeda* (Spirurida, Thelaziidae) in wolves (*Canis lupus*) in Italy. *J. Wildl. Dis.* 43(3),
202 508–511.
203 Otranto, D., Cantacessi, C., Testini, G., Lia, R.P., 2006b. *Phortica variegata* as an
204 intermediate host of *Thelazia callipaeda* under natural conditions: evidence for
205 pathogen transmission by a male arthropod vector. *Int. J. Parasitol.* 36, 1167–1173.
206 Otranto, D., Dantas-Torres, F., Mallia, E., DiGeronimo, P.M., Brianti, E., Testini, G.,
207 Traversa, D., Lia, R.P., 2009. *Thelazia callipaeda* (Spirurida, Thelaziidae) in wild
208 animals: report of new host species and ecological implications. *Vet. Parasitol*. 166 (3-
209 4), 262-267.
210 Otranto, D., Eberhard, M.L., 2011. Zoonotic helminths affecting the human eye. *Parasit*
211 *Vectors*. 23, 4, 41.
212 Otranto, D., Ferroglio, E., Lia, R., Traversa, D., Rossi, L., 2003a. Current status and
213 epidemiological observations of *Thelazia callipaeda* (Spirurida, Thelaziidae) in dogs,

cats and foxes in Italy: a “coincidence” or a parasitic disease of the Old Continent? *Vet. Parasitol.* 116, 315–325.

Otranto, D., Lia, R.P., Buono, V., Traversa, D., Giangaspero A., 2004. Biology of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms in naturally infected definitive host. *Parasitology* 129, 627–633.

Otranto, D., Lia, R.P., Cantacessi, C., Testini, G., Troccoli, A., Shen, J.L., Wang, Z.X., 2005a. Nematode biology and larval development of *Thelazia callipaeda* (Spirurida, Thelaziidae) in the drosophilid intermediate host in Europe and China. *Parasitology*. 131, 847–855.

Otranto, D., Lia, R.P., Traversa, D., Giannetto, S., 2003b. *Thelazia callipaeda* (Spirurida, Thelaziidae) of carnivores and humans: morphological study by light and scanning electron microscopy. *Parassitologia* 45(3-4), 125–133.

Otranto, D., Testini, G., De Luca, F., Hu, M., Shamsi, S., Gasser, R.B., 2005b. 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. *Mol. Cell. Probes* 19(5), 306–313.

Otranto, D., Traversa, D., 2005. Thelazia eyeworm: An original endo and ecto-parasitic nematode. *Trends Parasitol*. 1, 1–4.

Rodrigues, F.T., Cardoso, L., Coutinho, T., Otranto, D., Diz-Lopes, D., 2012. Ocular thelaziosis due to *Thelazia callipaeda* in a cat from northeastern Portugal. *J. Feline Med. Surg.* 14(12), 952-954.

Skrjabin, K.I., Sobolev, A.A., Ivashkin, V.M., 1967. Principles of nematology Vol. IX. Spirurata of animals and man and the disease caused by them. Part.4: Thelazioidea. Pub. by Israel Program for Scient. Transl. 1-54.

Vieira, L., Rodrigues, F.T., Costa, A., Diz-Lopes, D., Machado, J., Coutinho, T., Tuna, J., Latrofa, M.S., Cardoso, L., Otranto, D., 2012. First report of canine ocular thelaziosis by *Thelazia callipaeda* in Portugal. *Parasit Vectors* 5(1), 124.

Figure 1. Anterior end of female *Thelazia callipaeda* with vulva anterior to the oesophago-intestinal junction.

Figure 2. Large post- and pre-cloacal papillae at the posterior end of male *Thelazia callipaeda*.

Figure 3. Posterior end of male *Thelazia callipaeda* with long and short spicule.

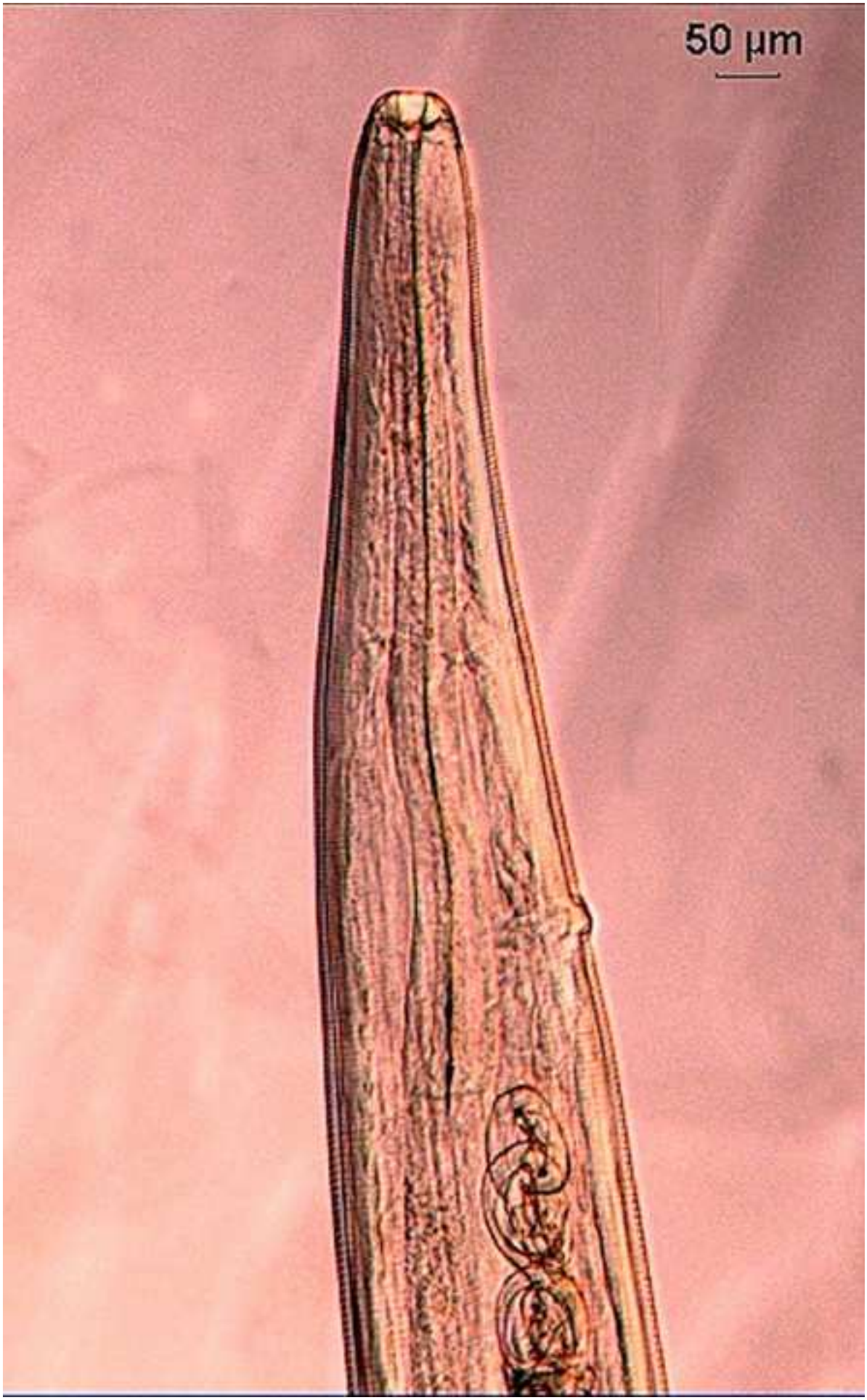

Figure 2