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11 **Spreading of *Thelazia callipaeda* in Greece**

12

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3 **Short title:** Thelaziosis in Greece

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9 **Summary**

10 *Thelazia callipaeda* (Spirurida, Thelaziidae), the so-called oriental eyeworm, has been
11 increasingly reported as an agent of infection in animals and humans from many
12 European countries. Clinical signs range from subclinical to moderate or severe ocular
13 disorders (e.g. epiphora, photophobia, conjunctivitis, keratitis, ulcers). The disease has
14 been also diagnosed in animals from countries of the Balkan area (e.g., Croatia, Bosnia
15 and Herzegovina and Serbia), but only a single case of canine thelaziosis, presumably
16 autochthonous, was reported in Northern Greece. In this study we provide robust
17 information of the occurrence of thelaziosis in Greece by reporting autochthonous cases
18 of thelaziosis in dogs (n =46), cats (n =3) and in one rabbit (*Oryctolagus cuniculus*)
19 living in Northern and Central regions of Greece. The occurrence of a single haplotype
20 of the cytochrome oxidase subunit 1 gene confirms that the same zoonotic haplotype of
21 the parasite circulating in Europe is also spreading in Greece. The increased awareness
22 of this parasitosis is crucial in order to limit the risk of further infections in both humans
23 and animals in European countries.

24

25 **Keywords** *Thelazia callipaeda*, eyeworm, dog, cat, rabbit, ocular, Greece

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28 **1. Introduction**

1 Adult nematodes of *Thelazia callipaeda* (Spirurida, Thelaziidae) inhabit the ocular
2 tissues of the eyes of animals and humans, mainly dwelling under the third eyelid of
3 animals (Otranto and Traversa, 2005). In the infected animals, the mechanical damage
4 and the load of adults and first-stage larvae (L1) are responsible for clinical signs
5 ranging from subclinical to moderate or severe ocular disorders (e.g. epiphora,
6 photophobia, conjunctivitis, keratitis, ulcers) (Shen et al, 2006; Otranto et al, 2013).
7 Though dogs and foxes are the predominantly infected hosts, also cats, hares and
8 wolves have been reported as suitable definitive hosts of *T. callipaeda* (Otranto et al,
9 2009). In Europe, the vector and intermediate host of this spirurid is the male
10 drosophilid fly *Phortica variegata* (Drosophilidae, Steganinae), which displays a
11 zoophilic behaviour feeding around the eyes of mammals (Otranto et al, 2006a, b). Over
12 the last decade, *T. callipaeda* has been increasingly reported from domestic animals and
13 wildlife in several European countries including Italy, France, Switzerland, Germany,
14 Spain and Portugal (Otranto et al, 2015). Human infections are mainly documented
15 from people living in East Asian countries (e.g. Japan, Korea, China, India) (Colwell et
16 al, 2011), though reports of ocular infection from humans in Italy, France, Spain, Serbia
17 and Croatia strengthen the medical importance of this parasitic infection also in
18 European countries (Otranto and Dutto, 2008; Fuentes et al, 2012; Paradžik et al, 2016;
19 Tasić-Otašević et al, 2016).

20 Recently, an increase in *T. callipaeda* infections has been reported in animals and
21 humans living in Eastern European countries (Colella et al, 2016). In Greece
22 information on *T. callipaeda* is limited to a single case report (presumably
23 autochthonous) from an infected dog living close to the borders with the former
24 Yugoslavian Republic of Macedonia (Diakou et al, 2015). In this study we provide
25 information of the occurrence, the clinical presentation and the treatment of a large case
26 series of thelaziosis in dogs, cats and a domestic rabbit (*Oryctolagus cuniculus*) living in
27 Northern and Central regions of Greece.

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29 **2. Materials and methods**

30 **2.1 Clinical examination and parasite collection**

31 From 2014 to 2016, a total of 50 client owned animals (46 dogs, 3 cats and 1 rabbit)
32 were referred to different veterinary practitioners due to ocular problems or for routine
33 veterinary examination. After administration of topical anaesthetic eye drops of

1 Proparacaine hydrochloride 0.5% (Alcaine®, ALCON, Belgium), animals were
2 subjected to ocular examination and the parasites were collected from the conjunctival
3 sac using fine forceps, sterile cotton swabs or flushing with saline solution. A brief
4 history of each animal was recorded including data on breed, age, gender, location,
5 lifestyle, clinical presentation and treatment.

6 **2.2 Morphological and molecular identification**

7 Nematodes collected from the eyes of infected animals were morphologically identified
8 at species level based on previous descriptions (e.g., shape of the buccal capsule,
9 presence of transversally striated cuticle and cloacal papillae, morphology of the
10 spicules in males, and the position of the vulva in females) (Skrjabin et al, 1971;
11 Otranto et al, 2003). The identification of the recovered specimens was further
12 confirmed by molecular analysis. Briefly, genomic DNA was extracted using a
13 commercial kit (DNeasy Blood & Tissue Kit, Qiagen, GmbH, Hilden, Germany) and a
14 partial sequence of the mitochondrial cytochrome oxidase subunit 1 (*cox1*) gene (689
15 bp) was amplified by PCR as previously described (Otranto et al, 2005). The amplicons
16 were purified and sequenced using the Taq Dye Doxy Terminator Cycle Sequencing Kit
17 (v.2, Applied Biosystems) in an automated sequencer (ABI-PRISM 377). Sequences
18 were compared with those available in GenBank database by Basic Local Alignment
19 Search Tool (BLASTn – <http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

20 **2.3 Ethic Statement**

21 All medical procedures were carried with the owner's approval. Nematodes were sent to
22 the laboratory of Parasitology (Aristotle University of Thessaloniki and University of
23 Bari) for morphological and molecular characterization.

24 **3. Results**

25 All infected animals (n=50) were located in 11 different areas of Northern and Central
26 Greece (i.e., Thessaloniki, Drama, Serres, Grevena, Kilkis, Edessa/Giannitsa, Veroia,
27 Chalkidiki, Kalampaka, Larisa and Athens - Figure 1). Animals were of both sexes,
28 aging from 0.6 to 11 years old with a history of irregular administration of endo- and
29 ecto-parasiticides (Table 1). None of them had travelled abroad or spent some period
30 close to bordering countries. Ophthalmological examination revealed the presence of
31 motile nematodes in the conjunctival sac and surrounding tissues of one or both eyes

1 (Figure 2). The number of removed nematodes ranged from 6 to 80 (mean= 29.4; sd=
2 ± 23.2), even though nematode counting was not possible in 10 out of 50 cases. All
3 animals presented ocular signs of various severity, except for two dogs and one cat.
4 Nematodes collected were morphologically identified as *T. callipaeda*. The *cox1*
5 sequences obtained from nematodes collected from all animal species were identical to
6 the sequence of *T. callipaeda* haplotype-1 (h1) (GenBank accession no. AM042549).

7 Based on clinical presentation and the decision of the clinician, different drugs were
8 administered (orally and/or locally) to reduce the gravity of ocular signs. Treatment
9 consisted in antibiotics (e.g., tobramycin, doxycycline, chloramphenicol) and cortico-
10 /steroids (dexamethasone, prednisolone; Table 1). In order to kill any remaining
11 parasites, ivermectin was administered in some animals (off label use of VALANEQ
12 injectable solution for ruminants, PNG Gerolymatos, Greece). The treatment, in some
13 cases, was completed with the monthly administration of antiparasitic combinations of
14 imidacloprid 10% and moxidectin 2.5% spot on (Advocate®, Bayer Animal Health,
15 Germany) or milbemycin oxime/praziquantel tablets (Milbemax®, Novartis-Animal
16 Health, France). Details on the lifestyle, sex, age, breed, main presenting reason, ocular
17 findings and treatment of infected animals are presented in Table 1.

18 19 **4. Discussion**

20 The large case series of infection by *T. callipaeda* in dogs, cats and a rabbit from
21 Northern and Central Greece, clearly indicate that the infection is rapidly spreading
22 throughout the country. This is in line with the increase in the number of reports of
23 thelaziosis in animals and humans living in Eastern European countries, including
24 Romania (Mihalca et al, 2015), Bosnia and Herzegovina (Hodžić et al, 2014), Croatia
25 (Hodžić et al, 2014; Paradžik et al, 2016), Serbia (Tasić-Otašević et al, 2016), Hungary
26 and Bulgaria (Colella et al, 2016). Indeed, except for a supposedly autochthonous case
27 report from a dog in 2015 (the parasite was not molecularly sequenced; Diakou et al,
28 2015), at the best of authors' knowledge, *T. callipaeda* has never been diagnosed before
29 in Greece. The occurrence of 50 autochthonous cases of thelaziosis in dogs, cats and in
30 a rabbit from several locations of Northern and Central Greece, indicates that *T.*
31 *callipaeda* is present among animals living in both urban and rural areas. However,
32 pathways associated with the introduction of *T. callipaeda* in Greece are not yet fully
33 understood. For instance, hunting dogs travelling from endemic areas of Italy and
34 France have been indicated as one of the possible route for the introduction of this

1 parasite in Spain (Mirò et al, 2011). Alongside, the occurrence of the vector and
2 intermediate host *P. variegata* has not yet been studied in Greece, though clinical cases
3 were reported from animals living in rural forested and meadow areas with a low
4 altitude (e.g. Kalambaka 244m, Kilkis 280m and Polygyros 426m above sea level),
5 which represent suitable ecotopes for this drosophilid (Otranto et al, 2006b; Mâca and
6 Otranto, 2014). Nonetheless, three infected dogs (Figure 1) lived in Athens, which is
7 farther to the south of Greece with altitude ranging from 70m to 338m above sea level.
8 In this study, the occurrence of *T. callipaeda* reached its southernmost point in Europe
9 (39°N/21°E and 37.9°N/23.7°E). The recent economic crisis in Greece may have
10 favoured the establishment and spread of *T. callipaeda* in animal populations, due to the
11 possible decrease in number of routine antiparasitic treatments of pets.

12 Furthermore, the role of wildlife, particularly foxes and wolves, as spreader of the
13 infection (Hodžić et al, 2014; Otranto et al, 2015) would deserve investigations. For
14 example, wolves have been recovered recently in Greece, which represents the south-
15 eastern edge of their distribution in Europe (Karamanlidis et al, 2016). In addition,
16 genetic studies on a population of wolves from Greece revealed the existence of a high
17 genetic variability (Karamanlidis et al, 2016) similar to those found in the Balkan area
18 (Moura et al, 2014). This data suggests that wolves roam and breed among bordering
19 countries, and may favour the introduction of pathogens, including *T. callipaeda*, in
20 suitable areas as suggested for Italy (Otranto et al, 2007). The participation of rabbits in
21 the maintenance of thelaziosis should be further assessed as reported in highly endemic
22 areas of Portugal (Gama et al, 2016).

23 Treatments of eyeworm infection include milbemycin oxime/praziquantel tablets (Motta
24 et al, 2012; Otranto et al, 2016) and imidacloprid 10% and moxidectin 2.5% spot-on
25 formulation (Lechat et al, 2015; Otranto et al, 2016), which showed to be effective
26 against *T. callipaeda* in naturally infected animals. In this study, treatment of thelaziosis
27 included macrocyclic lactones (in some cases off label use of ivermectin) and
28 symptomatic therapy, which was successful in clearing the infection and in a fully
29 recovering of animals from the associated clinical signs. Therefore, the treatment of
30 domestic animals with proper and registered products together with an increased
31 medical and veterinary communities' awareness of thelaziosis is central to limit further
32 infections in both animals and humans in Europe.

33 34 **Conflict of interest**

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

2

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8 **References**

- 9 Colella, V., Z. Kirkova, É. Fok, A.D. Mihalca, S. Tasić-Otašević, A. Hodžić, F. Dantas-
10 Torres, and D. Otranto, 2016: Increase in eyeworm infections in Eastern Europe.
11 *Emerg. Infect. Dis.* 22, 1513-1515.
- 12 Colwell, D.D., F. Dantas-Torres, and D. Otranto, 2011: Vector-borne parasitic
13 zoonoses: emerging scenarios and new perspectives. *Vet. Parasitol.* 182, 14-21.
- 14 Diakou, A., A. Di Cesare, S. Tzimoulia, I. Tzimoulias, and D. Traversa, 2015: *Thelazia*
15 *callipaeda* (Spirurida: Thelaziidae): first report in Greece and a case of canine infection.
16 *Parasitol. Res.* 114, 2771-2775.
- 17 Fuentes, I., I. Montes, J.M. Saugar, S. Latrofa, T. Garate, and D. Otranto, 2012:
18 Thelaziosis in humans, a zoonotic infection, Spain, 2011. *Emerg. Infect. Dis.* 18, 2073-
19 2075.
- 20 Gama, A., I. Pires, M. Canado, T. Coutinho, A.P. Lopes, M.S. Latrofa, L. Cardoso, F.
21 Dantas-Torres, and D. Otranto, 2016: First report of *Thelazia callipaeda* infection in
22 wild European rabbits (*Oryctolagus cuniculus*) in Portugal. *Parasite Vect.* 9, 236.
- 23 Hodžić, A., M.S. Latrofa, G. Annoncia, A. Alic, R. Beck, and R.P. Lia, 2014: The
24 spread of the zoonotic *Thelazia callipaeda* in the Balkan area. *Parasite Vect.* 7, 328.
- 25 Karamanlidis, A.A., S.D. Czarnomskac, A. Kopatz, L. Georgiadis, and B. Jedrzejewska,
26 2016: Wolf population genetics at the south-eastern edge of their European range.
27 *Mammalian Biol.* 81, 506–510.
- 28 Lechat, C., N. Siméon, O. Pennant, L. Desquilbet, S. Chahory, C. Le Sueur, and J.
29 Guillot, 2015: Comparative evaluation of the prophylactic activity of a slow-release
30 insecticide collar and a moxidectin spot-on formulation against *Thelazia callipaeda*
31 infection in naturally exposed dogs in France. *Parasite Vect.* 8, 93.

- 1 Màca, J., and D. Otranto, 2014: Drosophilidae feeding on animals and the inherent
2 mystery of their parasitism. *Parasite Vect.* 7, 516.
- 3 Mihalca, A.D, G. D'Amico, I. Scurtu, R. Chirilă, I.A. Matei, and A.M. Ionică, 2015.
4 Further spreading of canine oriental eyeworm in Europe: first report of *Thelazia*
5 *callipaeda* in Romania. *Parasite Vect.* 8, 48.
- 6 Mirò, G., A. Montoya, L. Hernandez, D. Dado, M.V. Vazquez, M. Benito, M.
7 Villagràsa, E. Brianti, D. Otranto, 2011: *Thelazia callipaeda*: infection in dogs: a new
8 parasite for Spain. *Parasite Vect.* 4, 148.
- 9 Motta, B., M. Schnyder, F. Solari Basano, F. Nægeli, C. Nægeli, B. Schiessl, E. Mallia,
10 R.P. Lia, F. Dantas-Torres, and D. Otranto, 2012: Therapeutic efficacy of milbemycin
11 oxime/praziquantel oral formulation (Milbemax®) against in naturally infested dogs
12 and cats. *Parasite Vect.* 19, 85.
- 13 Moura, A.E., E. Tsingarska, M.J. Dabrowski, S.D. Czarnomska, B. Jedrzejewska, and
14 M. Pilot, 2014: Unregulated hunting and genetic recovery from a severe population
15 decline: the cautionary case of Bulgarian wolves. *Conserv. Genet.* 15, 405–417.
- 16 Otranto, D., V. Colella, G. Crescenzo, F. Solari Basano, R. Nazzari, G. Capelli, G.
17 Petry, R. Schaper, M. Pollmeier, E. Mallia, F. Dantas-Torres, and R.P. Lia, 2016:
18 Efficacy of moxidectin 2.5% and imidacloprid 10% in the treatment of ocular
19 thelaziosis by *Thelazia callipaeda* in naturally infected dogs. *Vet. Parasitol.* 227, 118-
20 121.
- 21 Otranto, D., F. Dantas-Torres, E. Brianti, D. Traversa, D. Petrić, C. Genchi, and G.
22 Capelli, 2013: Vector-borne helminths of dogs and humans in Europe. *Parasite Vect.* 16,
23 16.
- 24 Otranto, D., G. Testini, F. Deluca, M. Hu, S. Shamsi, and R.B. Gasser, 2005: Analysis
25 of genetic variability within *Thelazia callipaeda* (Nematoda: Thelazioidea) from Europe
26 and Asia by sequencing and mutation scanning of mitochondrial cytochrome c oxidase
27 subunit I. *Mol Cell Probes* 19, 306-313.
- 28 Otranto, D., and D. Traversa, 2005: *Thelazia* eyeworm: an original endo- and ecto-
29 parasitic nematode. *Trends Parasitol.* 21, 1-4.
- 30 Otranto, D., C. Cantacessi, E. Mallia, and R.P. Lia, 2007: First report of *Thelazia*
31 *callipaeda* (Spirurida, Thelaziidae) in wolves in Italy. *J Wildlife Dis.* 43, 508-511.
- 32 Otranto, D., C. Cantacessi, F. Dantas-Torres, E. Brianti, M. Pfeffer, C. Genchi, V.
33 Guberti, G. Capelli, and P. Deplazes, 2015. The role of wild canids and felids in

1 spreading parasites to dogs and cats in Europe. Part II: Helminths and arthropods. Vet.
2 Parasitol. 213, 24-37.

3 Otranto, D., and M. Dutto, 2008: Human thelaziasis, Europe. Emerg. Infect. Dis. 14,
4 647-649.

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

8 Otranto, D., C. Cantacessi, G. Testini, and R.P. Lia, 2006a. *Phortica variegata* as an
9 intermediate host of *Thelazia callipaeda* under natural conditions: evidence for
10 pathogen transmission by a male arthropod vector. Int. J. Parasitol. 36, 1167–1173.

11 Otranto, D., E. Brianti, C. Cantacessi, R.P. Lia, and J. Máca, 2006b. Morphology,
12 ecology and biological niches of the zoophilic fruitfly, *Phortica variegata*. Med Vet
13 Entomol. 20, 358-364.

14 Otranto, D., F. Dantas-Torres, E. Mallia, P.M. DiGeronimo, E. Brianti, G. Testini, D.
15 Traversa, and R.P. Lia, 2009: *Thelazia callipaeda* (Spirurida, Thelaziidae) in wild
16 animals: report of new host species and ecological implications. Vet. Parasitol. 166,
17 262-267.

18 Paradžik, M., K. Samardžić, T. Živičnjak, F. Martinković, Z. Janjetović, and M.
19 Miletić-Medved, 2016: *Thelazia callipaeda*-first human case of thelaziosis in Croatia.
20 Wien Klin Wochenschr. 128, 221-223.

21 Shen, J., R.B. Gasser, D. Chu, Z.X. Wang, X. Yuan, C. Cantacessi, and D. Otranto,
22 2006: Human thelaziosis—a neglected parasitic disease of the eye. J Parasitol. 92, 872–
23 875.

24 Skrjabin, K.I., A.A. Sobolov, and V.M. Ivashkin, 1971: Essentials of nematodology,
25 Vol.16- Spirurata of animals and man and the diseases caused by them, part 4,
26 Thelazioidea. Israel Program for Translations, ISBN 978-0706511796, Jerusalem,
27 Israel.

28 Tasić-Otašević, S., S. Gabrielli, M. Trenkić-Božinović, A. Petrović, B. Gajić, V.
29 Colella, S. Momčilović, G. Cancrini, and D. Otranto, 2016: Eyeworm infections in dogs
30 and in a human patient in Serbia: A One Health approach is needed. Comp. Immunol.
31 Microbiol. Infect. Dis. 45, 20-22.

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1 **Figure 1:** Geographical location (dots) and number (within brackets) of infected
2 animals in Greece (D=dog, C=cat, R=rabbit).

3

4 **Figure 2:** *Thelazia callipaeda* eyeworms in the eye of an infected dog from Greece
5 (animal anesthetized).

6

7 **Table 1:** Details on the cases of animals infected with *Thelazia callipaeda* from
8 different areas of Greece.

Table 1: Details on the cases of animals infected with *Thelazia callipaeda* from different areas of Greece.

Animal Species	Lifestyle	Sex	Age (years)	Breed	Main presenting reason	Ocular findings (Mean number of nematodes \pm sd)	Treatment
Dog (n=46)	I/O/O+I	Male (n=26) Female (n=20)	0.6-11	Mongrel and breeds (Beagle, Boxer, German Shepherd, Greek hound, Griffon, Labrador retriever, Setter)	Ocular discharge with or without irritation, Conjunctival congestion, Incidental finding during physical examination or Routine vaccination	Conjunctivitis, mucoid or mucopurulent ocular discharge, follicular conjunctivitis, indolent corneal ulcer, corneal abrasions, blepharitis, anterior uveitis (37.02 \pm 21.48)	Tobramycin 0.1%+diclophenac 0.3% coll, TID x 10 D, Tobramycin 0.3%+Dexamethasone 0.1% coll, TID x 10D, Doxycycline 10 mg/kg po SID 28 D, chloramphenicol 0.5%+dexamethasone 0.1% coll, TID x 10 D, milbemycin oxime 0.9 mg/kg + praziquantel 9 mg/kg p.o. monthly, milbemycin oxime 1mg/kg + praziquantel 10 mg/kg p.o. monthly, 10% imidacloprid + 2.5% moxidectin spot on monthly,

							Ivermectin (400µg/kg, sc), Physical removal of nematodes
Cat (n=3)	I/O/O+I	Male (n=2) Female (n=1)	1.5-5	Greek shorthair	Ocular discharge- irritation, Routine vaccination	Conjunctivitis, No clinical signs (8.33 ±5.5)	Tobramycin 0.3%+Dexamethasone 0.1% coll, TID x 10D, Tobramycin 0.3% coll, Physical removal of nematodes
Rabbit (n=1)	O	Male	8.5		Ocular discharge	Conjunctivitis, mucoid ocular discharge (n=6)	Physical removal of nematodes

Lifestyle: O=outdoor, I=indoor

p.o.= per os, sc=subcutaneously, BID= bis in die, TID= ter in die (i.e. three times a day), D=day



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