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Occurrence of the Nematode *Thelazia callipaeda* Railliet and Henry, 1910 (Spirurida, Thelaziidae) in Wild Carnivores in the Russian Far East

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Abstract: Occurrence of the nematode *Thelazia callipaeda* Railliet and Henry, 1910 parasitising eyes of wild carnivores in the Russian Far East is studied. The hosts recorded in the course of the study are sable, *Martes zibellina* (492 studied, 28 infected); racoon dog, *Nyctereutes procyonoides* (11 studied, 5 infected); fox, *Vulpes vulpes* (3 studied, 2 infected); Asiatic black bear, *Ursus thibetanus* (1 studied, 1 infected; lynx (*Lynx lynx*) (1 studied, 1 infected). Sable, Asiatic black bear and lynx are new host records. Canids are considered the principal hosts for this parasite species. Morphology of *T. callipaeda* is studied and a short diagnostic description with morphometrics is given based on specimens from sable. Special attention is given to the arrangement of sensory structures of the male posterior end. The typical pattern is seven pairs of precloacal papillae, two pairs of paracloacal papillae and four pairs of postcloacal papillae. However, the papillae number and their position demonstrate substantial variation that prevents the reliable use of this character in the species diagnostic of the genus *Thelazia.*

Key words: parasitic eye infection, *Thelazia callipaeda,* carnivores, sable

Introduction

Thelazia callipaeda Railliet and Henry, 1910 (Spirurida, Thelaziidae) is a spirurid nematode parasitic in eyes of carnivores and humans. Its usual site of infection is in the conjunctival sac causing medium to severe inflammation of conjunctiva such as conjunctivitis, keratitis and ulcers of cornea (Otranto, Traversa 2005). Rarely, an intraocular localization was observed (Zakir *et al*. 1999, Xue *et al*. 2007, Chen *et al*. 2010). *Thelazia callipaeda* undergoes a heteroxenic life-cycle where a drosophilid fly *Amiota* (= *Phortica*) *variegata* serves as the nematode vector (Kozlov 1963a). The parasite is widely distributed in South and East Asia from Pakistan to Japan (Anderson 2000, Shen *et al*. 2006, Otranto, Eberhard 2011). In this region, the helminth is of significant medical importance and hence is known as an oriental eye worm. Since the first find in China in 1917, more than 250 cases of human infection by *T. callipaeda* were reported by 2000 (Faust 1928, Koyama *et al*. 2000), with 371 cases recorded in China only by 2006 (Shen *et al*. 2006).

Nowadays, the *T. callipaeda* infection ceased to be exclusively the Asian phenomenon. Starting in 1989, it has been reported in wild and domestic animals in Central and Southern Europe, i.e. in Italy, France, Switzerland, Spain, Portugal (Otranto *et al.*

2013), Germany (Magnis *et al*. 2010) as well as in Southeast Europe, i.e. in Bosnia and Herzegovina and Croatia (Hodžić *et al*. 2014). Later, the infection was discovered affecting humans in Europe (Otranto, Dutto 2008, Fuentes *et al*. 2012, Otranto *et al.* 2013). It was shown that in Europe the infection is autochtonous with the transmitting agent a local population of flies (Otranto *et al.* 2006).

Wild and domestic carnivores are principal hosts for *T. callipaeda*, maintaining the vital density of a parasite population. The list of definitive hosts of the parasite includes dogs (*Canis familiaris*), wild cats (*Felis silvestris*), domestic cats (*Felis catus*), wolfs (*Canis lupus*), foxes (*Vulpes vulpes*), racoon dogs (*Nyctereutes procyonoides*) and beech martens (*Martes foina*). In experiment, rabbits and monkeys were also infected (Faust 1928, Anderson 2000).

Within the Russian Federation, the infection of *T. callipaeda* was reported in dogs, cats, foxes and racoon dogs in the Russian Far East (Dehterev 1930, Kozlov 1963b, Oshmarin 1963). Two cases of infection in humans in this area were also described, namely in Khabarovsk Kray and Vladivostok (Kozlov 1963c, Miroshnichenko *et al.* 1988). However, no data of the occurrence of the parasite in Russian Federation for the period over 50 years are available.

Materials and Methods

Wild carnivores sampled in Primorskiy Kray (45°20′N 134°40′E) were necropsied in the period from the winter 2012 to the summer 2014. A single examined specimen of lynx was obtained in the Jewish Autonomous Oblast (48°36′N 132°12′E) in March 2014.

Conjunctival sacs of the animals including the space under a nictitating membrane were examined for the presence of thelaziids (Fig. 1).

Fig. 1. *Thelazia callipaeda* in eye of fox

Totally, 544 animals were examined. The host species belonged to the family Mustelidae, including 492 specimens of sables (*Martes zibellina)*, 25 Siberian weasels (*Mustela sibirica*), 4 American minks (*Neovison vison*), 2 yellow-throated martens (*Martes flavigula*) and one European badger (*Meles meles*); Canidae including 11 racoon dogs (*Nyctereutes procyonoides*) and 3 foxes (*Vulpes vulpes*); Felidae: 3 Amur leopard cats (*Prionailurus bengalensis euptilurus*) and one lynx (*Lynx lynx*); Ursidae: one brown bear (*Ursus arctos*) and one Asian black bear (*U. thibetanus*). Hosts names and classification is given according to Wilson, Reeder (2005).

To determine infection rate, the prevalence and mean abundance were used as described by Bush *et al.* (1997). Morphology was studied on temporary glycerine slides using light microscope Zeiss Axio Imager Z.1. The measurements were taken with the help of a digital camera attached to the microscope and associated software. The species was identified based on the morphology using for comparison the descriptions by Faust (1928), Dehterev (1929), Hsü (1933) and Furukawa (1981). Additional data on morphology presented by Bhaibulaya *et al.* (1970), Ryang *et al.* (1999), Otranto *et al.* (2003) were also used. Details of copulatory apparatus of males were studied on cut-off male tail ends. Measurements were taken from the specimens from sables (ten males and ten females). Morphometrics are presented as the range, mean and standard deviation (SD). Nematodes were preserved in 5 % formalin solution in 0.9 % NaCl and kept in the K.I. Skriabin Institute for Fundamental and Applied Parasitology of Animals and Plants, Moscow.

Results

Parameters of infection

Overall, 6.8% (37 out of 544) of the examined animals were infected by *T. callipaeda*. Infected animals were sables, racoon dogs, foxes, the Asian black bear and the lynx. In contrast, Siberian weasels, American minks, Amur leopard cats, yellow-throated martens, the brown bear and the badger were not infected (Table 1).

Morphological data

All collected nematodes were identified as *T. callipaeda* after comparing our morphological observations with previous descriptions (Faust 1928, Dehterev 1929, Hsü 1933, Furukawa 1981). No morphological differences between specimens from various hosts were observed. Since the sable is reported for the first time as the host for this nematode

Table 1. Occurrence of *Thelazia callipaeda* infection in wild carnivores in the Russian Far East. N: number of hosts examined; N+: number of hosts infected; * no value because only a single animal was examined

Host species	N	N+	Prevalence (%)	Intensity		Mean abundance
				Range	Mean	
Sable *Martes zibellina* L.	492	28	5.7	1-6	2.2	0.12
Racoon dog *Nyctereutes procyonoides* L.	11	5	45.5	1-5	2.4	1.09
Fox *Vulpes vulpes* L.	3	2	66.7	2-5	3.5	2.33
Asian black bear *Ursus thibetanus* L.	1	1	*	*	*	(3)
Lynx *Lynx lynx* L.	1	1	*	*	*	(1)

species, we provide below a brief description of the most important morphometric characters based on specimens from this host species.

Thelazia callipaeda Railliet & Henry, 1910

Host species: *Martes zibellina*

Site of infection: conjuctival sacs (Figs 2-7; Table 2).

Medium-sized nematodes, males c. 1 cm, females c. 1.5 cm long. Buccal capsule of characteristic shape for the genus, with unevenly thickened walls. Internal contour of buccal capsule vase-shaped (Fig. 2a). Cuticle annulated along whole body. Annulations seen as transverse striations. Cuticle with conspicuous folded transverse striations, spaced 4 µm apart at anterior body end to 6 µm at mid-body in males, and 5 to 8 µm, respectively, in females. Cuticular folds ornamented with numerous fine longitudinal ridges resembling spinules (Fig. 2b). Presence and position of ridges not constant.

Male: Spicules distinctly unequal, left one nearly 2 mm long, i.e. ten-fold longer than right one. Gubernaculum and caudal alae absent. Tail curved ventrally, with numerous ventral papillae (Fig. 3). Precloacal papillae roughly arranged in pairs in two rows; their number varying between five and eight; sometimes position of papillae irregular - single papilla or pairs of papillae can be displaced in lateral or longitudinal direction. Two pairs of paracloacal papillae, typically symmetrical, positioned laterally the level of cloaca. Among postcloacal papillae, most anterior pair at distance from others; second and third pairs located at same level, closely to one another, with papillae of lateral pair being larger than those of median one. Fourth (most posterior) pair of papillae situated in more lateral position. Phasmids large, exceed papillae in size, positioned ventrolaterally, close to tail tip. Position of phasmids and most posterior pair of papillae stable. Position of papillae around cloaca, including paracloacal ones, last precolacal pair and first postcloacal one may vary and exhibits various combinations. E.g., unpaired ventral median precloacal papilla presented, which can be interpreted as variation. Reduction of number of papillae was also observed.

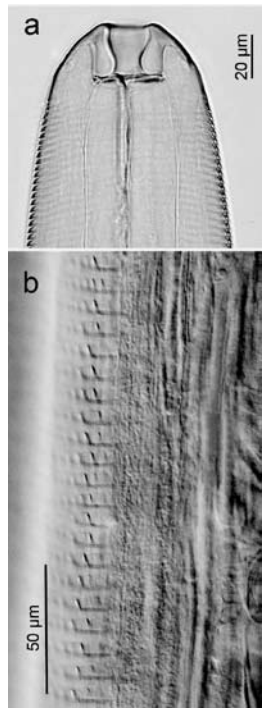

Fig. 2. Details of morphology of *Thelazia callipaeda* from sable from Primorskiy Kray. a – anterior end of male, lateral view, b – detail of cuticle

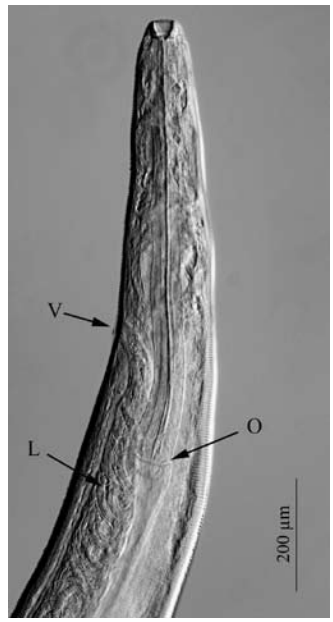

Fig. 3. Details of morphology of *Thelazia callipaeda* from sable from Primorskiy Kray. Female anterior end, lateral view. V – vulva, O – oesophago-intestinal junction, L – larvated eggs in vagina

Female: Vulva opens anterior to oesophago-intestinal junction. Mature eggs in uteri thin-shelled, containing fully developed first-stage larva (Fig. 4). Tail rounded. Phasmids conspicuous, subterminal (Fig. 5).

Table 2. Morphometrics of *Thelazia callipaeda* from sables from Primorskiy Kray, Russia. Measurements in micrometers unless otherwise stated

Measurements	Males (N=10)			Females (N=10)		
	Range	Mean	SD	Range	Mean	SD
Body length, mm	9.4-11.5	10.2	0.6	11.5-15.4	14.3	1.3
Body width at mid-body	284-314	298	11.3	280-392	340	33.9
Distance from anterior end to deirids	374-450	409	25.1	428-503	461	26.5
Maximum width of buccal capsule	33-45	40	4.3	43-50	46	2.9
Length of buccal capsule	23-29	26	2.2	31-34	33	1.5
Minimum thickness of buccal capsule wall	6-8	7	0.9	6-7	7	0.5
Maximum thickness of buccal capsule wall	8-12	10	1.4	9-11	10	0.6
Oesophagus, length	573-655	614	27.5	646-790	739	47.9
Length of right spicule	121-150	135	10.6	-	-	-
Length of left spicule	1555-1962	1788	134.2	-	-	-
Distance from anterior end to vulva	-	-	-	436-594	549	50.0
Distance from vulva to the end of the oesophagus	-	-	-	188-269	226	28.3
Tail length	74-95	84	8.6	63-98	78	11.1

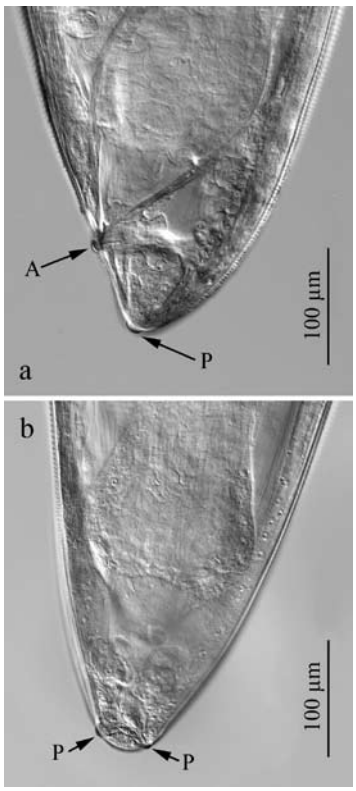

Fig. 4. Details of morphology of *Thelazia callipaeda* from sable from Primorskiy Kray. Posterior end of female. a – lateral view, b – ventral view. A – anus, P – phasmids

Discussion

Our study expands the data of the host range of *T. callipaeda*. Sable, Asian black bear and wild lynx are new host records of this species. Until present, *T. callipaeda* infection in lynx has been registered only in captivity in a zoo (El-Dakhly *et al*. 2012). This addition to the list of the host species confirms the presence of the wide host range of the parasite species, which differentiates it from the majority of the members of the genus *Thelazia* which are characterised by the high host specificity.

The parameters of infection of *T. callipaeda* varied in different hosts. Both prevalence and mean abundance in racoon dogs and foxes were distinctly higher than in sables. This fact corresponds well with the known data indicating on the higher infection rate of domestic and wild canids compared with other mammal hosts (Otranto *et al.* 2003, Hodžić *et al*. 2014) and points out that canids should be considered as the principal hosts for *T. callipaeda*. The nature of the host specificity of *T. callipaeda* is poorly known. We assume that it can be associated with the physiological factors determining the parasite survival in hosts, as well as the ecological and behavioural peculiarities of hosts providing its contacts with flies.

Our own data, as well as previous studies (Dehterev 1930, Kozlov 1963b, Oshmarin 1963) show a presence of a persistent natural focus of *T. callipaeda* in the Russian Far East, which potentially can threaten the epidemiological and epizootiological situation in the area. Our results also demonstrate that presently the infection of *T. callipaeda* is extending its distribution on major parts of the Eurasian continent, involving new host species in its life cycle. The causes of this phenomenon remain unclear.

Concerning the morphology of the studied specimens, our observations are interesting from the point of view of morphology of the posterior end of

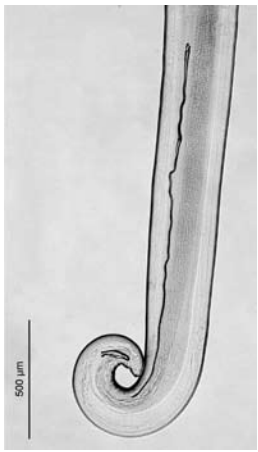

Fig. 5. Details of morphology of *Thelazia callipaeda* from sable from Primorskiy Kray. Male posterior end, lateral view

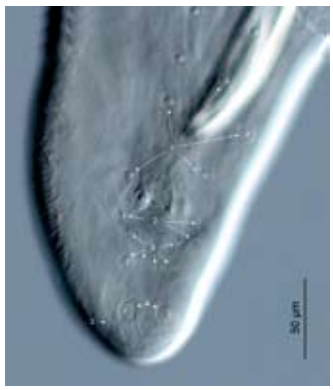

Fig. 6. Male caudal papillae of *Thelazia callipaeda* from sable from Primorskiy Kray. Designation of papillae: 1 – precloacal, 2 – paracloacal, 3 – postcloacal (note irregularity of arrangement in precloacal papillae and the absence of one paracloacal papilla); 4 – phasmid

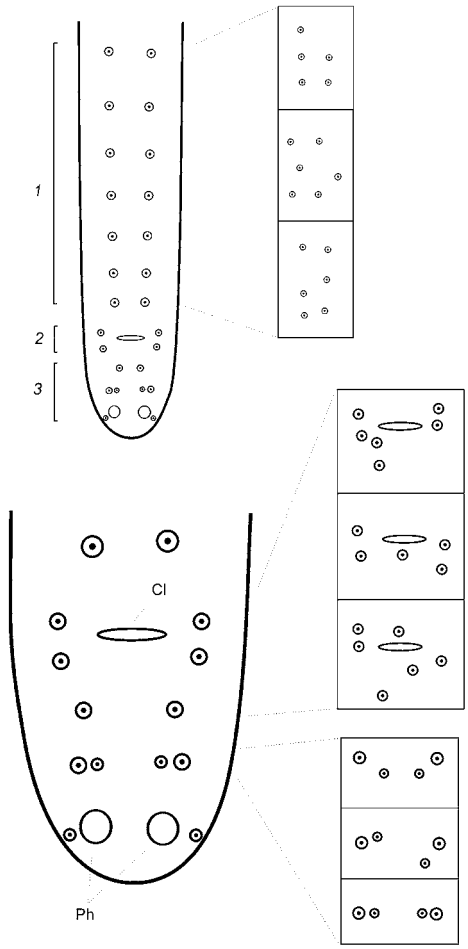

Fig. 7. Diagram showing the position of male caudal papillae of *Thelazia callipaeda*.
Papillae: 1 – precloacal, 2 – paracloacal, 3 – postcloacal. Cl – cloacal aperture, Ph – phasmids. Inserts to precloacal papillae: illustrate variation in some contiguous pairs of papillae that can be placed in any part of the region. Inserts to pericloacal zone: notice high range of variability of papillae position breaking their symmetrical arrangement

males. The organisation of male caudal papillae is a typical characteristic for spirurid nematodes and its peculiarities are mentioned in standard species description. However, the data on the number and position of male caudal papillae in *T. callipaeda* are controversial. Faust (1928) noted the presence of six-eight pairs of precloacal papillae and two pairs of postcloacal ones; Dehterev (1929, 1930) reported in one case the presence of 12 and two pairs, respectively and in another case nine and five; Hsü (1933) counted eight -10 and five pairs. The data obtained with the use of scanning electron microscopy also were in discordance about the number of papilae. For example, according to Bhaibulaya *et al.* (1970), there were ten pairs of precloacal and five pairs of postcloacal papillae, while Furukawa (1981) reported eight pairs of precloacal, a pair of adcloacal, four pairs of postclocal including a pair located closely to tail tip. According to Ryang *et al.* (1999), a full complement of male caudal papillae comprised eight pairs of precloacal papillae and three-four pairs of postcloacal ones. Kim *et al.* (2002) accounted total 24±2 papillae, including seven pairs precloacal and five postcloacal ones; Otranto *et al.* (2003) respectively 15 pairs with ten precloacal and five postcloacal, while Sohn *et al.* (2011) reported the presence of seven pairs of precloacal and two pairs of postcloacal papillae.

Our study showed that the number and position of male caudal papillae of *T. callipaeda* are highly variable and differ across nematode individuals. Moreover, the differentiation of precloacal and postcloacal papillae is hindered by the unstable position of papillae around cloaca. We assume that the typical complement of caudal papillae should comprise seven pairs of precloacal, two pairs of paracloacal and four pairs of postcloacal papillae. Additionally, there is a pair of phasmids located closely to tail tip, which were considered as papillae by the majority of authors (Fig. 6, 7). Such basic pattern of papillae arrangement was rarely observed in our material and should be considered as an ideal scheme with many variations which we have described above.

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References

Anderson R. C. 2000. Nematode parasites of vertebrates: their development and transmission. CABI. 650 p.

Bhaibulaya M., S. Prasertsilpa and S. Vajrasthira 1970. *Thelazia callipaeda* Railliet and Henry, 1910, in man and dog in Thailand. – *The American Journal of Tropical Medicine and Hygiene*, **19** (3): 476-479.

Bush A. O., K. D. Lafferty, J. M. Lotz and A. W. Shostak 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. – *Journal of Parasitology*, **83**: 575-583.

Chen W., J. Zheng, P. Hou, L. Li and Y. Hu 2010. A case of intraocular thelaziasis with rhegmatogenous retinal detachment. – *Clinical and Experimental Optometry*, **93** (5): 360-362.

Dehterev N. A. 1929. Ocular thelaziosis of a dog in Far East. – *Trudy Gosudarstvennogo instituta eksperimental'noj veterinarii*, **6** (1): 54-61 (in Russian).

Dehterev N. A. 1930. Ocular thelaziosis in dogs in USSR. – *Trudy Dalnevostochnogo instituta eksperimentalnoj veterinarii*, **6**: 133-138. (In Russian)

El-Dakhly K., S. Abo El-Hadid, H. Shimizu, S. El-Nahass, A. Murai, H. Sakai and T. Yanai 2012. Occurrence of *Thelazia callipaeda* and *Toxocara cati* in an imported European lynx (*Lynx lynx*) in Japan. – *Journal of Zoo and Wildlife Medicine*, **43** (3): 632-635.

Faust E. C. 1928. Studies on *Thelazia callipaeda* Railliet and Henry, 1910. – *The Journal of Parasitology*, **15**: 75-86.

Fuentes I., I. Montes, J. M. Saugar, S. Latrofa, T. Gárate and D. Otranto 2012. Thelaziosis in humans, a zoonotic infection, Spain, 2011. – *Emerging Infectious Diseases*, **18** (12): 2073-2075.

Furukawa K. 1981. Light microscopic and scanning electron microscopic observations on the *Thelazia callipaeda* (Railliet et Henry, 1910) infected in the dog conjunctiva. – *Medical Journal of Kinki University*, **6** (3): 303-317.

Hodžić A., M. S. Latrofa, G. Annoscia, A. Alic, R. Beck, R. P. Lia, F. Dantas-Torres and D. Otranto 2014. The spread of zoonotic *Thelazia callipaeda* in the Balkan area. – *Parasites and Vectors*, **7**: 352.

Hsü H. F. 1933. On *Thelazia callipaeda* Railliet et Henry, 1910 infection in man and dog. – *Archiv für Schiffs - und Tropen-Hygiene Leipzig,* **37**: 363—369.

Kim S., K. Joo and M. Chung 2002. Ultrastructural character on the cuticular surface of *Thelazia callipaeda*. – *Korean Journal of Electronic Microbiology*, **32**: 45-55.

Koyama Y., A. Ohira, T. Kono, T. Yoneyama and K. Shiwaku 2000. Five cases of thelaziasis. – *British Journal of Ophthalmology*, **84** (4): 441.

Kozlov D. P. 1963a. Biology of *Thelazia callipaeda* Railliet and Henry, 1910. – *Trudy Gelmintogischeskoi Laboratorii*, **13**: 330-346. (In Russian)

Kozlov D. P. 1963b. The study of the helminth fauna of animals from family Canidae of Far East. – *Trudy Gelmintogischeskoi Laboratorii*, **13**: 56-74. (In Russian)

Kozlov D. P. 1963c. First report of *Thelazia callipaeda* Railliet et Henry, 1910 in man in the USSR. – *Trudy Gelmintogischeskoi Laboratorii*, **13**: 75-77. (In Russian)

Magnis J., T. J. Naucke, A. Mathis, P. Deplazes and M. Schnyder 2010. Local transmission of the eye worm *Thelazia callipaeda* in southern Germany. – *Parasitology Research*, **106** (3): 715-717.

Miroshnichenko V. A., M. P. Desiaterik, A. P. Novik, A. P. Gorbach and N. Papernova 1988. A case of ocular thelaziasis in a 3-year-old child. – *Vestnik Oftal'mologii,* **2**: 64. (In Russian)

Oshmarin P. G. 1963. Parasitic worms of mammals and birds of Primorsky Krai. Moscow (Izdatel'stvo Academii Nauk SSSR). 323 p. (In Russian)

Otranto D., D. Traversa 2005. Thelazia eyeworm: an original endo- and ecto-parasitic nematode. – *Trends in Parasitology*, **21** (1):1-4.

Otranto D., M. Dutto 2008. Human thelaziasis, Europe. – *Emerging Infectious Diseases*, **14** (4): 647-649.

Otranto D., M. L. Eberhard 2011. Zoonotic helminths affecting the human eye. – *Parasites and Vectors*, **4**: 41.

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

Otranto D., C. Cantacessi, G. Testini and R. P. Lia 2006. *Phortica variegata* as an intermediate host of *Thelazia callipaeda* under natural conditions: evidence for pathogen transmission by a male arthropod vector. – *International Journal for Parasitology*, **36** (10-11): 1167-1173.

Otranto D., F. Dantas-Torres, E. Brianti, D. Traversa, D. Petric, C. Genchi and G. Capelli 2013. Vector-borne helminths of dogs and humans in Europe. – *Parasites and Vectors*, **6**: 16.

Ryang Y.-S., K.-J. Lee, D.-H. Lee, Y.-K. Cho, J.-A. Im, J.-Y. Park and H.-S. Han 1999. Scanning electron microscopy of *Thelazia callipaeda* Railliet and Henry, 1910 in the eye of a dog. – *Korean Journal of Biomedical Laboratory Sciences*, **5** (1): 41-49.

Shen J., R. B. Gasser, D. Chu, Z. Wang, X. Yuan, C. Cantacessi and D. Otranto 2006. Human thelaziosis--a neglected parasitic disease of the eye. – *The Journal of Parasitology*, **92** (4): 872-875.

Sohn W. M., B. K. Na and J. M. Yoo 2011. Two cases of human thelaziasis and brief review of Korean cases. – *Korean Journal of Parasitology*, **49** (3): 265-271.

Wilson D. E., D. A. Reeder 2005. Mammal species of the world. A taxonomic and geographic reference, 3rd edition. Baltimore, Maryland (Johns Hopkins University Press). 2142 p.

Xue C., N. Tian and Z. Huang 2007. *Thelazia callipaeda* in human vitreous. – *Canadian Journal of Ophthalmology*, **42** (6): 884-885.

Zakir R., Z. Zhong-Xia, P. Chioddini and C. R. Canning 1999. Intraocular infestation with the worm, *Thelazia callipaeda*. – *British Journal of Ophthalmology*, **83** (10): 1194-1195.

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