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Thelazia callipaeda (Spirurida, Thelaziidae) in wild animals: Report of new host species and ecological implications

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ABSTRACT

Thelazia callipaeda infects the eyes of carnivores and humans in Far Eastern Asiatic and European countries. Studies have demonstrated the occurrence of *T. callipaeda* in foxes from areas where canine thelaziosis is endemic. However, there is little information on the role of wild carnivores as hosts of this nematode. From May 2003 to May 2009, a total of 130 carcasses of red foxes (*Vulpes vulpes*; $n = 75$), wolves (*Canis lupus*; $n = 2$), beech martens (*Martes foina*; $n = 22$), brown hares (*Lepus europaeus*; $n = 13$), Eurasian badgers (*Meles meles*; $n = 10$), and wild cats (*Felis silvestris*; $n = 8$) were examined in an area of southern Italy where canine thelaziosis is highly prevalent. At necropsy, animals were examined and nematodes were collected from the conjunctival sacs of both eyes. All nematodes were morphologically identified and at least five specimens from each of the five host species were molecularly processed by PCR amplification and sequencing of a partial mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*). Five out of the six wild animal species examined were found to be infected with eyeworms. The overall infection rate, excluding the Eurasian badgers that were all negative, was 39.1%. All the 189 adult nematodes collected (intensity of infection = 4 ± 2.2) were morphologically identified as *T. callipaeda*. The molecular analysis confirmed that the only haplotype of *T. callipaeda* circulating in Europe (i.e., haplotype 1) is present in that area. The competence of red foxes, wolves, beech martens, brown hares, and wild cats as definitive hosts for *T. callipaeda* is discussed in relationship to their ecology and their likely exposure to the vector *Phortica variegata* in the study area. The role the wild fauna plays in maintaining and spreading eyeworm infection in humans and domestic animals is also discussed.

1. Introduction

Thelaziosis, also known as eyeworm infection, is caused by nematodes of the genus *Thelazia* (Spirurida: Thelaziidae), which are transmitted by secretophagous flies into the orbital cavities and surrounding tissues of many species of wild and domestic mammals (reviewed in Otranto and Traversa, 2005). Out of 16 species of *Thelazia* described so far, *Thelazia callipaeda* infects carnivores and humans and it has long been referred to as "oriental eyeworm" due to its occurrence in humans and dogs from the Russian Federation and the Far East (i.e., Indonesia, Thailand, China, Korea, Myanmar, India, and Japan) (Bhaibulaya et al., 1970; Kosin et al., 1989; Hong et al., 1995; Shen et al., 2006). This nematode species can also infect cats, foxes, rabbits (Kozlov, 1962; Skrjabin et al.,

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1967), and also wolves (Otranto et al., 2007). In affected animals, *T. callipaeda* adult and larval stages may cause mild ocular manifestations (e.g., conjunctivitis, epiphora, and ocular discharge) to severe (e.g., keratitis, and corneal ulcers) (Otranto and Traversa, 2005). In the past two decades, *T. callipaeda* has spurred the attention of the scientific community due to its spread in some European countries (i.e., Italy, France, and Switzerland) (Rossi and Bertaglia, 1989; Otranto et al., 2003; Dorchies et al., 2007; Malacrida et al., 2008) and to imported cases in previously free areas (i.e., Germany; Hermosilla et al., 2004). Furthermore, the first four cases of human thelaziosis in Europe have been diagnosed in northern Italy, in patients with history of travel to north-western Italy and south-eastern France (Otranto and Dutto, 2008).

The highest prevalence of canine thelaziosis has been reported in some areas of southern Italy (Basilicata region), reaching up to 60.14% in some municipalities (Otranto et al., 2003). In the same hyper-endemic area, a number of studies have been carried out to elucidate several aspects of the biology of *T. callipaeda* in definitive and intermediate hosts (Otranto et al., 2004a). *Phortica variegata* (Diptera: Drosophilidae) has been implicated as the vector of *T. callipaeda* under both experimental and field conditions (Otranto et al., 2005a, 2006b). A genetic screening of the hypervariable region of mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) of *T. callipaeda* indicated the existence of only one haplotype (designated as haplotype 1; i.e., h1) in Europe and seven haplotypes in Asia (Otranto et al., 2005b). The presence of a single haplotype of *T. callipaeda* in Europe, irrespective of country of origin (i.e., Italy, France, and Germany) and hosts (e.g., dogs, cats, and foxes) suggested a close association and a likely co-evolution between the nematode and its vector (Otranto et al., 2005b). This knowledge raised questions on the role of wild carnivores in maintaining the life cycle of *T. callipaeda* in nature (Otranto et al., 2006a). Studies have shown the occurrence of *T. callipaeda* in foxes in areas where canine thelaziosis is endemic, indicating that foxes could act as reservoirs of *T. callipaeda* (Rossi et al., 2002; Otranto et al., 2003; Malacrida et al., 2008). However, with the exception of wolves (Otranto et al., 2007), no data is available on the role of wild carnivores as hosts of *T. callipaeda*. Lack of knowledge on the sylvatic life cycle of *T. callipaeda* has constituted a foundation for the present study whose aim was to evaluate the occurrence of *T. callipaeda* in wildlife species that could be acting as definitive hosts and reservoirs for this nematode.

2. Materials and methods

2.1. Study area

The survey was carried out in the *T. callipaeda*-hyper-endemic area of the Basilicata region in southern Italy (latitude 39° and 41° North, longitude 15° and 16° East) which comprises 12 municipalities (Otranto et al., 2003). The area is situated at an altitude between 800 and 1000 m above sea level (a.s.l.) with an orography characterized by the presence of a ring of sandstone mountains surrounding the examined area. Vegetation characteristics may vary according to altitude, exposure to the sunlight, and relative humidity. In particular, oaks are common, with *Quercus cerris* being the most common species and holly (*Ilex* spp.) found in the undergrowth. The study area is included in the Regional Park "Gallipoli Cognato Piccole Dolomiti Lucane" which hosts many indigenous populations of protected animals including red foxes (*Vulpes vulpes*), wolves (*Canis lupus*), beech martens (*Martes foina*), brown hares (*Lepus europaeus*), Eurasian badgers (*Meles meles*), and European wildcats (*Felis silvestris*). Many dogs usually accompanying sheep and cattle at least once a day during summer are also present in the same area, in which, the population dynamics of *P. variegata* has been monitored demonstrating that these flies are mainly active in July–August at 20–25 °C and 50–75% of relative humidity (Otranto et al., 2006a).

2.2. Post-mortem examination and parasite collection

From May 2003 to May 2009, 130 carcasses of wild animals belonging to different species (Table 1) were examined in the study area. Animals were found within the park territory, killed by trauma (following impact with a motor vehicle) or illegally killed, and thus provided by the local authorities to determine the cause of death. All animals were delivered to the park's resident veterinarian (Dr. Egidio Mallia). Animals were kept frozen until necropsies were carried out based on the number of specimens retrieved in batches of about 10 animals.

At necropsy, animals were examined, their sex recorded and their age estimated. In particular they were categorized as juveniles or adults on the basis of biomorpho-

Table 1
Number (no.) and percentage (%) of different host species found infected by *Thelazia callipaeda* categorized by sex and age. Details about nematodes sex and location and mean intensity of infection are also provided. Data from Eurasian badgers ($n = 10$), which were all negative, have been omitted from the analysis.

Host species (no.)	Host data					Nematode data					
	Male	Female	Juvenile	Adult	Total (%)	Male	Female	Right eye	Left eye	Range (mean intensity ± SD)	Total
Vulpes vulpes (75)	20	17	12	25	37 (49.3)	42	97	82	57	1–13 (3.8 ± 2)	139
Canis lupus (2)	1	–	–	1	1 (50)	–	2	–	2	–	2
Martes foina (22)	1	2	3	–	3 (13.6)	6	9	9	6	3–6 (5 ± 1.7)	15
Lepus europaeus (13)	–	3	–	3	3 (23.1)	3	14	9	8	3–11 (5.7 ± 4.6)	17
Felis silvestris (8)	2	1	1	2	3 (37.5)	9	7	4	12	3–7 (5.3 ± 2.1)	16
Total (120)	24	23	16	31	47 (39.2)	60	129	104	85	1–13 (4 ± 2.2)	189