

## First Report of *Thelazia callipaeda* in Wildlife from Spain

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**ABSTRACT:** We describe the first cases of infection by the nematode, *Thelazia callipaeda* (Spirurida, Thelaziidae) haplotype 1 in two red foxes (*Vulpes vulpes*) in Spain and discuss the potential role of red foxes as a reservoir for *T. callipaeda*.

*Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms infect the orbital cavities and surrounding tissues of carnivores and humans, causing mild (conjunctivitis, epiphora, ocular discharge) to severe (keratitis, corneal ulcers) signs (Anderson, 2000). The nematode is transmitted by the fruitfly, *Phortica variegata* (Diptera, Drosophilidae), which deposits infective third-stage larvae in the conjunctival sacs while feeding on the lacrimal secretions of receptive hosts. Over the past two decades, thelaziosis associated with *T. callipaeda* has been found in dogs (*Canis lupus familiaris*) and cats (*Felis catus*) in several European countries including Italy, Switzerland, and Portugal (Otranto et al., 2003; Malacrida et al., 2008; Vieira et al., 2012) and was recently found in dogs in Spain (Miró et al., 2011). In addition, human cases of thelaziosis are reported in France, Italy, and Spain (Otranto and Dutto, 2008; Fuentes et al., 2012).

In all these studies, no evidence of genetic variability in *T. callipaeda* was detected in Europe, irrespective of country of origin or hosts (Otranto et al., 2005b), and only one haplotype (designated as haplotype 1; =h1) was identified by sequencing a hypervariable region of mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*). The presence of a single haplotype of *T. callipaeda* suggests a close association and a likely coevolution between the nematode and its vector (Otranto et al., 2005a).

In areas where canine thelaziosis is enzootic, *T. callipaeda* may occur in red foxes, suggesting that this species may act as a reservoir for the nematode (Malacrida et al., 2008; Otranto et al., 2009). More information on the epizootiology of the nematode in wild animals from areas where thelaziosis has been recently reported may help to understand its origin and development in wild reservoirs. We report the first cases of thelaziosis caused by *T. callipaeda* in red foxes from Spain.

In December 2011, two red foxes, a juvenile male (76 cm long, 44 cm thoracic circumference, 7.35 kg weight) and an adult female (71 cm, 44 cm, 7.15 kg, respectively), were shot by hunters in a free-hunting area in the center of Cáceres Province (39°32'32.4"N, 6°32'17.6"W), Extremadura region, in southwest Spain. The area is a semidesert Mediterranean ecosystem (430 m elevation, 700 mm annual rainfall, 18 C average annual temperature, mild Mediterranean climate) with vegetation consisting primarily of oaks (*Quercus rotundifolia*, *Quercus ilex*, and *Quercus suber*) and shrubs (*Cistus ladanifer* and *Cytisus scoparius*). A high prevalence of canine thelaziosis was recorded in the area (Miró et al., 2011).

At necropsy, adult nematodes were retrieved from the conjunctival sacs by flushing with saline solution (0.9% NaCl) and stored in 70% ethanol for morphologic and molecular identification. Nematodes were identified using the keys of Skrjabin et al. (1967) and Otranto et al. (2004). For molecular identification, the worms were subjected to specific PCR amplification of the 689 base-pair portion of the *cox1* gene (Otranto et al., 2005b), and amplicon sequences were determined



FIGURE 1. Adult specimen of the zoonotic nematode *Thelazia callipaeda* in the right eye of a male red fox (*Vulpes vulpes*) in Spain.

in both directions by visual inspection of the individual electropherograms. Sequences were aligned using Clustal X (Thompson et al., 1997) and the alignments were compared with sequences available in public databases for the *cox1* of *T. callipaeda*. Two female worms were found in the right eye of the male red fox (Fig. 1) and 12 (seven gravid females and two males in the right eye; three females in the left eye) in the female red fox. The morphologic identification as *T. callipaeda* h1 was confirmed molecularly.

This is the first report of thelaziosis in red foxes from Spain and confirms *T. callipaeda* presence in a wild species in the southern Spanish Mediterranean forest ecosystems, as has been seen in Switzerland and Italy (Malacrida et al., 2008; Otranto et al., 2009). In areas with a high prevalence in domestic dogs, *T. callipaeda* occurs in several wild species (e.g., wolf [*Canis lupus*], brown hares [*Lepus granatensis*], wild cats [*Felis silvestris*]; Otranto et al., 2009), suggesting circulation among various hosts. Cáceres province falls within the range of the geoclimatic provisional model for distribution of the arthropod vector *P. variegata* (Otranto et al., 2006a; Miró et al., 2011), although its presence has not been confirmed and further studies are required.

Our study demonstrates host competence of red foxes by retrieval of gravid female and mature male *T. callipaeda* in the eyes of one fox. This implies that third-stage larvae, from an appropriate vector, developed to adult nematodes in the ocular cavity. The retrieval of h1, the only haplotype present in Europe (Malacrida et al., 2008; Otranto et al., 2009; Miró et al., 2011; Vieira et al., 2012), further supports the hypothesis that a high degree of specificity of *T. callipaeda* for their vectors (Otranto et al., 2005a, 2006b), but not their definitive host, facilitates transmission, circulation, and spread of the nematode in areas where competent hosts occur.

The sylvatic life cycle of *T. callipaeda* in foxes in southwest Spain, an area with a high prevalence of canine thelaziosis, suggests foxes may act as potential reservoirs of *T. callipaeda* where pet carnivores are under veterinary control (Miró et al., 2011). Red fox habitat use fits with the ecologic characteristics of the vector in the same environment (Otranto et al., 2006a) and foxes, along with dogs, may serve as reservoirs for human infestation in Spain as was also suggested by the first human infection (Fuentes et al., 2012).

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