First reports of autochthonous eyeworm infection by Thelazia callipaeda (Spirurida, Thelaziidae) in dogs and cat from France

Ph. Dorchies a,*, G. Chaudieu b, L.A. Sime’on c, G. Cazalot a, C. Cantacessi d, D. Otranto d
a Ecole vétérinaire de Toulouse, 23 chemin des Capelles, 31076 Toulouse Cedex, France
b 2 place Beaulieu, 63400 Chalamie`res, France
c 247 route d’Angoule`me, 24000 Pe´rigueux, France
d Department of Animal Health and Welfare, Faculty of Veterinary Medicine, University of Bari, Italy
Received 27 June 2007; received in revised form 23 July 2007; accepted 1 August 2007

* Corresponding author. Tel.: +33 5 61 19 38 71; fax: +33 5 61 19 39 44. E-mail address:
p.dorchies@envt.fr (P. Dorchies).
0304-4017/$ – see front matter # 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2007.08.005

Abstract

Thelazia callipaeda (Spirurida, Thelaziidae) is a small nematode living in the conjunctival sac of domestic and wild carnivores, rabbits and humans causing lacrimation, epiphora, conjunctivitis, keratitis and even corneal ulcers. The first autochthonous cases of thelaziosis affecting four dogs and one cat living in SouthWestern France (Dordogne area) are reported and described. Nematodes recovered from the animals were morphologically identified as T. callipaeda and a partial region of the cytochrome oxidase c subunit 1 gene (cox1) was amplified by PCR from nematode specimens (from two dogs and the cat). In each case, this was shown to have an identical sequence to the haplotype 1 (h1) of T. callipaeda. So far, the arthropod acting as intermediate host of T. callipaeda eyeworms has not been identified in France although it might be Phortica variegata (Steganinae, Drosophilidae) as recently described in Italy.

Keywords: Thelazia callipaeda; Phortica variegata; Nematode; Dog; Cat; France; Europe

1. Introduction

Thelazia callipaeda (Spirurida, Thelaziidae) is a small nematode living in the conjunctival sac of domestic (i.e., dogs and cats) and wild carnivores (i.e., foxes and wolves), rabbits and humans causing lacrimation, epiphora, conjunctivitis, keratitis and even corneal ulcers (reviewed in Anderson, 2000; Otranto et al., 2007). It has been recently demonstrated, both under laboratory (Otranto et al., 2005a) and natural conditions (Otranto et al., 2006b) that in Italy this infection is transmitted by Phortica variegata (Steganinae, Drosophilidae) feeding on the lacrimal secretions of the definitive hosts. The distribution of this nematode, commonly known as “oriental eyeworm” has been considered for a long time to be confined to the former soviet republics and Asia (reviewed by Anderson, 2000) where it causes infections in humans, dogs and cats (reviewed by Shen et al., 2006). However, in the past decade thelaziosis caused by T. callipaeda has been
demonstrated to be widespread among dogs and cats from northern to southern Italy with infection prevalence up to 60.14% in dogs from some municipalities of southern Italy (Otranto et al., 2003). Cases of thelaziosis have also been registered sporadically in France (Chermette et al., 2004) and Germany (Hermosilla et al., 2004) in dogs which had spent some time, during summer, in northern Italy.

A molecular investigation employing a mitochondrial DNA marker (Otranto et al., 2005b) demonstrated that, despite a relatively high degree of genetic variability among T. callipaeda isolates from Asia, no genetic variation occurs between individual nematodes collected from different host species (i.e., dogs, cats and foxes) and localities within Europe (i.e., Italy, Germany and the Netherlands). This finding suggested a strict affiliation of this nematode to the intermediate host rather than to the definitive hosts and that the distribution of the parasite would be expected to coincide with that of the vector (Otranto et al., 2005b). In a recent work the ecological niche model suitable for P. variegata flies was predicted both for Italy and Europe, and the most suitable areas have been found to be concentrated in central Europe, particularly in France (Otranto et al., 2006a).

The present paper reports the first French autochthonous cases of thelaziosis caused by T. callipaeda in four dogs and one cat. Nematode larvae from these cases were morphologically and molecularly identified.

2 Materials and methods
2.1 Case reports

All the animals referred and described below had spent their lives in the Dordogne region, or had spent some time there during holidays, and they had never travelled abroad. Most of the cases came from a strawberry production area around the village of Vergt (latitude: 45°8'01''41''00''N; longitude: 0°8'43''09''09''E). This area is close to the Atlantic Ocean and it is included in Aquitaine (latitude: 44–45°N and longitude close to 0°). Its altitude ranges from 112 to 246 mt above sea level and it has an oceanic climate with an average of 800 mm annual rainfall. The vegetation is mixed.

Since March 2006, four dogs referred to veterinary practitioners with unilateral conjunctivitis were found on clinical examination to have adult nematodes in the conjunctival sac. In September 2006, a similar finding was made in the conjunctival sac of a 2-year-old male adult farm cat (Brown Tabby European) suffering from unilateral conjunctivitis (left eye) with severe chemosis and a wound on the 3rd eyelid (without corneal ulcer).

After removal of the worms by flushing with saline solution, all dogs and the cat were treated topically and received milbemycin per os (Milbemax1) as per label recommendations. No recidives were registered.

2.2. Morphological and molecular identification

Nematodes collected from the eyes of the above animals were stored in 70% ethanol and sent to the Parasitological Unit of the Faculty of Veterinary Medicine (University of Bari, Italy) for the morphological and molecular identification.

All nematodes collected were identified according the keys of Skrjabin et al. (1967) and Otranto et al. (2004). To confirm the morphological identification and to analyse the haplotype sequence, a total number of four specimens (from two dogs and the cat) were molecularly processed via the specific amplification by PCR of a partial sequence of the mitochondrial cytochrome oxidase subunit 1 gene (cox1—689 bp) as previously described (Otranto et al., 2005b). Briefly, genomic DNA from single nematodes was isolated using the QIAamp Tissue Kit (Qiagen GmbH, Germany) and eluted in 50 ml H2O. A partial sequence of the mitochondrial cox1 was amplified using primers NTF (5′-TGATTGTTGGTTTGTGAA-30′) and NTR(5′-GATATTGATACGTACGTATTT-30′) (Casiraghi et al., 2001). GenomicDNA (4 ml) was added to the PCR reaction mix (46 ml) containing 2.5 mM MgCl2, 10 mM Tris–HCl, pH 8.3 and 50 mM KCl, 250 mM of each dNTP, 50
pmol of each primer and 1.25 U of Ampli Taq Gold (Applied Biosystems). PCR was performed in an Applied Biosystems 2700 thermal cycler using the following cycling protocol: 94 °C for 12 min (polymerase activation), followed by 40 cycles of 95 °C for 1 min (denaturation); 48 °C for 1 min (annealing); 72 °C for 1 min (extension), followed by 7 min at 72 °C (final extension). Negative and positive control reactions were also carried out by substituting the Thelazia DNA template with sterile water and T. callipaeda DNA, respectively. Amplicons were purified using Ultrafree-DA columns (Amicon, Millipore; Bedford, MA, USA) and sequenced in a ABI-PRISM377 using the TaqDyeDeoxyTerminator Cycle Sequencing Kit (Applied Biosystems).

Sequences were determined in both directions (using the same primers individually as for the PCR) and the electro-pherograms verified by eye. Sequences were aligned using the ClustalX program (Thompson et al., 1997). The alignments were verified by eye and compared with the sequences available for the cox1 of T. callipaeda (Gen Bank accession nos. AM042549, AM04550, AM04551, AM04552, AM04553, AM04554, AM04555, AM04556; Otranto et al., 2005b).

3. Results and discussion

Five worms from dogs and one adult female from a cat have been morphologically identified as three male and three female T. callipaeda. The cox1 sequences obtained were identical to the sequence representing haplotype 1 (i.e., h1) (available in Gen Bank accession no. AM042549, see Otranto et al., 2005b). The clinical cases here described represent the first reports of autochthonous thelaziosis by T. callipaeda affecting French dogs and cats which had never travelled abroad. Previously Chermette et al. (2004) had described cases of canine thelaziosis in France, but this was in animals that had spent time during the summer in northern Italy where, the authors argued, infection by T. callipaeda eyeworms had occurred. The occurrence of adult T. callipaeda specimens on the conjunctiva of dogs and cats implies that third stage larvae, released by the insect intermediate host, had developed to adult nematodes in the ocular cavity, thus suggesting the presence of the arthropod vector in the area that infection occurred. In a paper dealing with the molecular delineation of isolates of T. callipaeda from different European and Asiatic localities, Otranto et al. (2005b) recorded an infected dog from the Netherlands that had earlier spent 3 months in the Dordogne region of southern France. The above report, along with the autochthonous cases here described, lend credit to the hypothesis that T. callipaeda is present in the Dordogne. This region is at the same latitude as Piedmont in northern Italy where canine thelaziosis has been previously reported (Rossi and Bertaglia, 1989). It also fits into the putative areas in Europe in which P. variegata flies (which act as vectors for T. callipaeda eyeworms in southern Italy) could be endemic based on a predictive geoclimatic model (Otranto et al., 2006a). In addition, the natural environment in which P. variegata flies have been collected in Italy matches to some extent with that of Dordogne region.

Interestingly, all autochthonous cases of thelaziosis here reported came from an environment close to strawberry farms where the intermediate host P. variegata, a drosophilid fruitfly, may develop (Otranto et al., 2006b). It has been reported that male P. variegata feed on tears and other animal secretions, while females grow and develop on fruit and other vegetable matter, thus making the environment in which T. callipaeda infections have been reported, highly suitable for the vector. However, further studies are needed in order to identify the species of arthropod acting as intermediate host in France.

The results of the present work indicate that T. callipaeda infection is present in France and that h1 is the only haplotype found in Europe. This raises questions about the origin of thelaziosis in France. It has been recently argued that wild fauna (i.e., foxes and wolves) play a major role in maintaining and spreading this nematode disease among pets in rural areas (Otranto et al., 2007). Considering the characteristics of the environment in which T. callipaeda has been found in France, the possibility cannot be excluded that canine thelaziosis has been introduced into this region by wild animals moving from northern Italy, from where fox thelaziosis has been previously reported (Rossi et al., 2002). Therefore, the autochthonous population of red foxes, which are spread across
the whole Dordogne region (reviewed by Aubert, 1999), may have contributed to the establishment of the infection by T. callipaeda in pets from that area. Despite the high number of cases of T. callipaeda infection registered across all Europe (Otranto et al., 2003; Chermette et al., 2004; Hermosilla et al., 2004), no human case has yet been reported. This may be because a poor awareness of the zoonotic potential of this parasite among physicians across Europe could lead to underdiagnosis of the human disease, which is easily confused with bacterial and viral conjunctivitis (reviewed by Shen et al., 2006).

References


