

Thelazia eyeworm: an original endo- and ecto-parasitic nematode

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The genus *Thelazia* comprises several parasites, commonly named eyeworms, which infect the eyes and associated tissues of mammals, including humans. Transmission of eyeworms occurs via non-biting diptera that feed on the ocular secretions, tears and conjunctiva of animals. The disease, thelaziosis, is characterized by a range of sub-clinical to clinical signs, such as epiphora, conjunctivitis, keratitis, corneal opacity and ulcers. Human thelaziosis is common in poor socio-economic settings in many Asian countries. The relationship between eyeworms and their hosts are discussed here, together with recent molecular insights that are instrumental in investigating the biology of *Thelazia* in their definitive and intermediate hosts.

Nematodes belonging to the genus *Thelazia* (Spirurida, Thelaziidae), commonly known as eyeworms, inhabit

the orbital cavities and associated tissues of several mammals, such as canids, felids, ruminants and equids, including humans [1–3]. Of the 16 different species of *Thelazia* found worldwide [1,2] (Table 1), six of them (*Thelazia californiensis*, *Thelazia callipaeda*, *Thelazia gulosa*, *Thelazia lacrymalis*, *Thelazia rhodesi* and *Thelazia skrjabini*) are frequently retrieved and are of veterinary and/or medical concern. Two of these species, *T. callipaeda* and *T. californiensis*, infect humans.

The intermediate hosts of *Thelazia* are non-biting diptera (Box 1). Adults of *Thelazia* spp. can be found under the lids and nictitating membrane, in nasolachrymal ducts, conjunctival sacs or in the excretory ducts of lachrymal glands, according to the *Thelazia* species [2].

Table 1. *Thelazia* species, hosts, vectors and their geographic distribution

Species	Hosts parasitized	Vectors	Geographic distribution	Refs
<i>Thelazia brevispiculata</i>	Cattle	Unknown	China	[2]
<i>Thelazia bubalis</i>	Buffaloes	Unknown	India	[1]
<i>Thelazia californiensis</i>	Canids, felids, domestic and wild ruminants, humans	<i>Fannia benjamini</i> , <i>Fannia canicularis</i> ^a	North America	[1–3]
<i>Thelazia callipaeda</i>	Canids, felids, rodents, humans	<i>Amiota okada</i> ^a , <i>Amiota variegata</i> ^a	Italy, ex-USSR, Far East countries	[2,21,23]
<i>Thelazia depressa</i>	Mustelids	Unknown	Africa	[1]
<i>Thelazia erschowi</i>	Pigs	Unknown	Ex-USSR	[1,2]
<i>Thelazia ferulata</i>	Cattle	Unknown	China	[2]
<i>Thelazia gulosa</i>	Domestic and wild ruminants	<i>Musca amica</i> , <i>Musca autumnalis</i> , <i>Musca domestica</i> ^b , <i>Musca larvipara</i> , <i>Musca osiris</i> , <i>Musca vitripennis</i>	Europe, Asia, North America, Australia	[1–3,16,19]
<i>Thelazia hsüi</i>	Cattle	Unknown	China	[2]
<i>Thelazia iheringi</i>	Agouties	Unknown	South America	[2]
<i>Thelazia kansuensis</i>	Cattle	Unknown	China	[2]
<i>Thelazia lacrymalis</i>	Equids, domestic and wild ruminants	<i>M. autumnalis</i> ^a , <i>M. osiris</i>	Europe, North and South America	[2,20]
<i>Thelazia leesei</i>	Camels	<i>Musca lucidula</i>	Ex-USSR, India	[2,3]
<i>Thelazia petrowi</i>	Cattle	Unknown	Ex-USSR	[2]
<i>Thelazia rhodesi</i>	Domestic and wild ruminants, horses	<i>M. autumnalis</i> , <i>Musca convexifrons</i> , <i>Musca crassirostris</i> , <i>M. domestica</i> ^b , <i>Musca hervei</i> ^a , <i>M. larvipara</i> , <i>Musca sorbens</i>	Europe, Asia, Africa, North and South America	[1–3,6,16,19]
<i>Thelazia skrjabini</i>	Domestic and wild ruminants	<i>M. amica</i> , <i>M. autumnalis</i> ^a , <i>M. hervei</i> ^a , <i>M. osiris</i>	Europe, Asia, North America, Australia	[1–3,18]

^aUnder experimental conditions.

^bThe role of *Musca domestica* as intermediate host of *Thelazia gulosa* and *Thelazia rhodesi* is still controversial (see Refs [15,19] for details).

Box 1. The life cycle of *Thelazia*

Both the females and males adult eyeworms inhabit under the lids and nictitating membrane, in naso-lachrymal ducts, conjunctival sacs and excretory ducts of the lachrymal glands of animals (Figure 1a). Transmission occurs by means of secretophagous non-biting flies which feed on animal lachrymal secretions and become infected with the first stage larvae (L1) (Figure 1b–c) that are released in the lachrymal secretions of the hosts by adult females, after mating with the adult males. The larvae undergo developmental stages from the L1 to the infective third-stage larvae (L3), while remaining encapsulated in different parts of the vector's body (Figure 1d), depending on the *Thelazia* species. The L3s emerge from the labella of infected flies (Figure 1e), then feed on the lachrymal secretions of animals and develop into the adult stage in the ocular cavity. Figures 1d and e are reproduced, with permission, from Ref. [18]. Scale bars: 70 μ m (b); 750 μ m (d); 440 μ m (e).

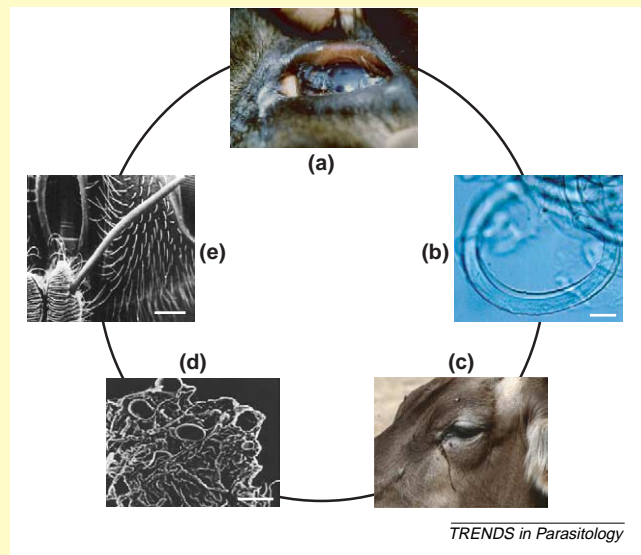


Figure 1.

Relationship between *Thelazia* and their definitive hosts

The biology and epidemiology of *Thelazia* eyeworms are influenced by the susceptibility of their definitive hosts, and mainly by the presence, biology and seasonality of their vectors. Bovine thelaziosis is the most commonly reported animal thelaziosis, and has been broadly studied in North America and Europe probably because of the susceptibility of cows to *Thelazia* and that free-grazing animals are more likely to be exposed than other animals (e.g. carnivores) to fly vectors of the eyeworm.

The high prevalence and intensity of infection in adult (rather than in young) cattle and dogs have been explained by: (i) the long lifespan of *Thelazia* (more than one year); (ii) the likely absence of a protective immunological response; and (iii) re-infections of the parasite which can occur throughout the animal's life [2,4,5]. Despite the presence of adult parasites throughout the year, there is a pattern in the dynamics of parasitism for *Thelazia* in cattle, with two peaks: one in early summer (adults that overwinter) and one in late summer (adults developing from infectious stages laid by the vectors in early summer) [6]. In the UK, the number of immature *Thelazia* stages in cattle increases in the summer

concomitantly with the disappearance of adults, suggesting that there might be a phenomenon occurring similar to the self-cure phenomenon of gastrointestinal strongyles in sheep [4].

First-stage larvae (L1) of *T. callipaeda* have been found in the lachrymal secretions of naturally infected dogs during the summer season probably because the larvae are ready to be ingested by flies feeding around the eyes. The detection of fourth-stage larvae (L4) of *T. callipaeda* in spring and early fall, however, is consistent with the presence of intermediate hosts during these periods and with overlapping generations of nematodes [7] because L4s could originate from L3s released by flies in the spring and/or late summer to early fall.

The adult and larval stages are responsible for eye disease, with symptoms of varying degrees of severity, including lacrimation, ocular discharge, epiphora, conjunctivitis, keratitis, corneal opacity or ulcers (Figure 1; Figure 1a). Asymptomatic, subclinical thelaziosis occurs mainly when only the male nematodes parasitize animals, whereas evident symptoms have been more frequently registered in the presence of gravid females, thus indicating the important role larvae play in the pathogenesis of conjunctivitis [8,9]. The lateral serration of the *Thelazia* cuticle causes mechanical damage to the conjunctival and corneal epithelium, resulting in ocular discharge, which is of significance for worm transmission as flies feed on lachrymal secretions containing L1s.

The symptoms of bacterial bovine keratitis by *Moraxella bovis* (pinkeye) are similar to those of thelaziosis and both diseases occur when secretophagous flies are present. The incidence of pinkeye increases proportionally to the fly population density (flies carry *M. bovis*) and pinkeye most likely develops on traumatic lesions caused by *Thelazia* spp. (for review, see Ref. [10]).

Since the first report of human thelaziosis from Beijing, China [11], the number of human cases has increased in the Far East [12], whereas autochthonous cases have never been reported in Europe. The occurrence of human thelaziosis has been related to: (i) the presence of parasitized dogs in the same environment [12,13]; (ii) the age of the individuals (the elderly and children aged between three and six years represent the most commonly infected individuals, accounting for 61% of human thelaziosis); and (iii) weather conditions (in China, 74% of cases occur in summer and fall, when flies are abundant and farmers leave their children under shady trees [14]).

Relationship between *Thelazia* and their intermediate hosts

Over the past few years, the number of surveys on the epidemiology of *Thelazia* in the intermediate hosts have been limited by the difficulties in retrieving larvae from vectors because of the low prevalence and mean intensity of infected flies [15]. Investigations on the vectors of *Thelazia* have been carried out on species affecting cows in North America, Slovakia and the ex-USSR by dissecting experimentally and naturally infected flies. Among several species, *Musca autumnalis* and *Musca larvipara* (commonly named face flies) have been incriminated in the transmission of eyeworms [6,10,15–18]. The detection of

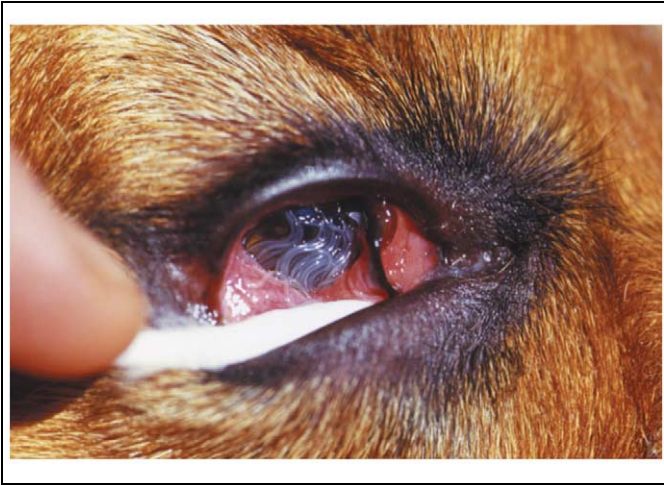


Figure 1. Eye of a dog with heavy infection by *Thelazia* spp. Reproduced, with permission, from Ref. [5].

Thelazia third-stage larvae (L3) in the intermediate hosts is particularly challenging because it is difficult to dissect flies and identification to species is only possible by comparing larval eyeworms recovered from laboratory infected flies [15,17–19].

A molecular epidemiological survey, carried out to identify three bovine *Thelazia* species in the fly vectors in field conditions in southern Italy, revealed a mean infection rate of 2.86% in the whole fly population collected throughout five seasons and assessed the role of different *Musca* species as intermediate hosts [19]. Unfortunately, there is little information available on the intermediate hosts of *T. lacrymalis*, which infect equids. Most of the information is on the immature stages in *Musca osiris* (see Ref. [2] for details) and the development of larvae to L3 in experimentally infected *M. autumnalis* [20].

Although thelaziosis by *T. callipaeda* is of public health concern in Asian countries, the natural vector/s of this parasitic worm is not known and its life cycle in the intermediate host is poorly understood. There is some evidence that *Musca domestica* (Diptera, Muscidae), and *Amiota variegata* and *Amiota okadai* (Diptera, Drosophilidae) could be involved as vectors of *T. callipaeda* in the Asia Pacific regions [21–23]. The recent report of thelaziosis in dogs, cats and foxes in northern and southern Italy raises several issues concerning the provenance of *T. callipaeda*. In particular, whether thelaziosis caused by *T. callipaeda* in Italy is autochthonous or has been imported from the ex-USSR and/or Asian countries where it is believed to be confined [5]. In this case, molecular studies are instrumental in understanding the origin of *T. callipaeda* in Europe. Furthermore, knowledge of the vector/s species involved in transmission of *T. callipaeda* is of paramount importance in clarifying its epidemiology in Europe and Asia.

Molecular insights

Over the past 20 years, extensive studies on some target genes [i.e. ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA)] have provided tools for identifying parasitic nematodes, and for the study of their systematics, epidemiology and biology irrespective of their

developmental stages [24]. The ribosomal internal transcribed spacer 1 (ITS1) sequence of *T. callipaeda*, *T. gulosa*, *T. lacrymalis*, *T. rhodesi* and *T. skrjabini* has been characterized to advance our knowledge of the rDNA in Spirurida (for which little information is available compared with that for the Ascaridida and Strongylida Orders). In addition, these data will assist in the taxonomy and molecular identification of these nematodes in their definitive and intermediate hosts [25].

One of the most interesting features of the *Thelazia* ITS1 is that the length of this sequence differs substantially from one species to the other, unlike for other non-spirurid parasitic nematodes ranked within the same genus. The interspecific size polymorphism, together with the high AT content and the presence of microsatellites within the ITS1 sequences, represent distinct characteristics of spirurids [25]. The occurrence of microsatellites leads to intraspecific variation in the length of the ITSs in eyeworms [25] and also in nematodes of other Spirurida (e.g. *Mansonella ozzardi*, *Onchocerca volvulus* [26] and *Habronema* spp. [27]). The polymorphism of ITS1 has proved to be important for the simultaneous identification of bovine *Thelazia* species by a PCR–RFLP assay [28].

Concluding remarks

Thelazia spp. probably represent one of the most extraordinary taxons among parasitic nematodes because of their relationship with definitive and intermediate hosts. In the definitive host, *Thelazia* are endoparasitic nematodes living in the anterior eye chamber and thus exposed to the air and the outside environment, just like ectoparasites. Because of the eyeworm's habitat, thelaziosis is the only nematode infection that can be treated topically, by direct instillation of drugs into the eyes. *Thelazia*, together with few other nematodes within the Spirurida Order (i.e. *Habronema* spp., *Stephanofilaria* spp. and *Parafilaria* spp.), are the only nematodes transmitted by secretophagous flies [10]. The life cycle of *Thelazia* eyeworms in their intermediate hosts is an extraordinary example of capitalization by *Thelazia* of the flies' feeding habits, so that the parasites can be transmitted into the eyes of the definitive hosts. Hence, the relationship between *Thelazia* and flies represents an interesting model of co-evolution of nematodes and insects and, more generally, an example to improve our understanding of the interaction between parasites and their vectors.

Reliable molecular identification of *Thelazia* spp. is of importance in controlling eyeworm infection in definitive hosts, particularly as some species can cross-infect the same animals and different species have different susceptibilities to anthelmintic drugs. Molecular identification of larval eyeworms in their vectors is also instrumental for studying the epidemiology of *Thelazia*, its prevalence in flies and the role of different insect species as intermediate hosts. The use of molecular approaches to study eyeworms overcomes the many constraints during insect dissection – a laborious, time-consuming process with low levels of sensitivity. Finally, the occurrence of microsatellite repeats in the ribosomal sequences could be a powerful tool for investigating the genetic make-up of

T. callipaeda isolates from different geographical origin. This information will help us understand the origin of thelaziosis, as with *T. callipaeda* in Europe, and whether the parasite is from an autochthonous or imported foci. Molecular data on the bovine *Thelazia* will also be useful to gain further insight into the epidemiology of *T. callipaeda* [19,25,28], considering that the rapid change of habitats and temperatures induces modifications in the dipteran fly population and in arthropod-borne parasitoses.

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