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***Thelazia* Species and Conjunctivitis**

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1. Introduction

Thelazia nematodes (Spirurida: Thelazioidea), are commonly known as eyeworms and cause ocular infections in animals and humans (Anderson 2000; Soulsby 1986). This genus of spirurids represents one of the most specific taxon among nematodes because of its very close relationship with its intermediate and final hosts (Otranto and Traversa 2005). *Thelazia* Bosc, 1819 should be considered an “endoparasite”. However, the immature and mature stages occur in the anterior chamber of the eye, thereby being exposed to the external environment. Therefore, it could be considered an “ectoparasite”. The sixteen species of this genus have been reported from canids, felids, ruminants, equids and humans (Skrjabin et al. 1971; Yamaguti 1961). They have been documented in Europe (Italy, France, Switzerland and Germany), Asia (China, Japan, Korea and Taiwan), North America (Canada and U.S.A.), South America (Peru) and South Africa. The adult worms live under the eyelids, nictitating membranes and lacrimal ducts. However, they are also found in the nose and pharynx. They are milky-white worms, with males measuring up to 12 mm in length and females up to 18 mm in length. The numbers of pre-cloacal and post-cloacal papillae in males differ among species. Usually, the spicules are unequal. Also, the location of the vaginal opening and the number of cuticular transverse striations differ among species. The worms are viviparous, and the first-stage larvae are passed by females into the lachrymal secretions where they are ingested by non-biting Diptera flies. Larval development takes place in the thorax and abdomen of the vector, and infective stages are present in 18–25 days. Development to the adult stage takes place without migration, and the prepatent period is between 3 and 6 weeks. The first stage larva of *Thelazia* is very short-lived in the lachrymal secretions, only surviving a few hours, and transmission depends upon the continuous presence of the vectors. For this reason, thelaziasis has a seasonal occurrence according to the seasonality of the intermediate hosts (Dunn 1978). The two species known to cause human thelaziasis are *T. callipaeda* and *T. californiensis*. *T. callipaeda* is commonly found in humans and animals (dogs, cats, foxes, wolves and rabbits) in the former Soviet Union, China, South Korea, Japan, Indonesia, Thailand Taiwan, and India (Anderson 2000), while *T. californiensis* is found in the United States (YJ Yang et al. 2006). Although human ocular parasitic infestation is rare, additional case studies are needed to fully understand the route of infestation and pathogenic mechanism. In final hosts, both the larval stages and adults of *Thelazia* spp. cause clinical signs such as excessive lacrimation, epiphora, conjunctivitis, keratitis and corneal ulcers (Hong et al. 1995; Kim, 2010; Otranto and Dutto, 2008; Singh and Singh 1993; CH Yang et

al. 2005). A definitive diagnosis is made by the detection of the parasites in the conjunctival sac. Examination of lacrimal secretions may reveal eggs or first-stage larvae. Also, morphological differentiation has been done on some *Thelazia* species using scanning electron microscopy (WY Choi et al. 1989; Naem 2005, 2007a,b,c). Molecular characterization and phylogeny of some *Thelazia* species have been studied by Nadler et al. (2007), Otranto et al. (2001, 2005b), and Traversa et al. (2005). Due to the localization of the nematode, thelaziasis can be treated topically by direct application of drugs into the eyes. Removal of the adult parasites with fine forceps, using local anesthesia, is also helpful. Patients with an intraocular infestation with *T. callipaeda* have been successfully treated with a pars plana vitrectomy. Michalski (1976) found that two ml of levamisole injected into the subconjunctival sac was more effective than levamisole given orally. Treatment of dog thelaziasis, caused by *T. callipaeda*, using a topical formulation of 10% imidacloprid and 2.5% moxidectin has been studied by Bianciardi and Otranto (2005). Also, the prophylactic use of a monthly treatment with milemycin oxime showed a 90% efficacy against *T. callipaeda* in naturally exposed dogs (Ferroglia et al., 2008). In this chapter, both human and animal thelaziasis will be discussed.

2. Etiologic agent: *Thelazia* Bosc, 1819

Thelazia Bosc is parasite of the conjunctival sac or lacrimal ducts of mammals and birds. The presence of the nematodes in these particular sites is usually sufficient for a generic diagnosis. The adult worms are small, thin and milky-white in color. Females measure up to 20 mm in length, and males measure up to 12 mm in length. The mouth has no lips and the anterior border of the buccal cavity is everted and divided by indentations into six festoons, of which four appear to be occupied by a small refractile papilliform organ. There are two lateral and four submedian cephalic papillae. The body may be transversely striated. The male's tail is blunt, and has no caudal alae. There are many pre-cloacal papillae, one of which is unpaired and located in front of the anal pore and three or four pairs are post-cloacal. The spicules are unequal. The posterior end of the female is bluntly rounded, with a pair of lateral papillae near its end. The females are larviparous or viviparous and the vulva is in the esophageal region (Dunn 1978; Soulsby 1986). *Thelazia* species found in canids, felids, ruminants, equids, birds, and humans (Anderson 2000; Skrjabin et al. 1971; Yamaguti 1961).

3. Human thelaziasis

Very few cases of human thelaziasis are reported worldwide. There are two species of the genus *Thelazia* that have been found in the human eye, *Thelazia callipaeda* (Railliet and Henry 1910) and, more rarely, *Thelazia californiensis* (Price 1930). The former species occurs under the nictitating membrane of the eye in canids, cats, rabbits, rats, monkeys and, rarely, humans in China, Japan, India, Burma, Korea, Taiwan, Thailand, Indonesia, Russia, Italy, Switzerland, Germany, and France (Anderson 2000; Otranto and Eberhard 2011; Ruytoor et al. 2010; YJ Yang et al. 2006). *T. californiensis* is mainly a parasite of dogs, but it has also been reported in sheep, deer, coyotes, cats, bears, and rarely humans in western North America. Most ocular *T. californiensis* infestations in humans have occurred in California, particularly in the Sierra Nevada Mountains. However, this worm's habitat is also located within areas of New Mexico, Nevada, Arizona and Oregon (Anderson 2000; Levine 1968).

3.1 *Thelazia callipaeda* and *T. californiensis*

3.1.1 Morphology

T. callipaeda is nicknamed the “oriental eyeworm” because of its widespread prevalence in the Far East. Adult worms look like creamy white threads. Light and scanning electron microscopy have been used to study the surface of adult worms of this nematode (Arizono et al. 1976; DK Choi and Choi 1979; WY Choi et al. 1989; Kagei et al. 1983; Min and Chun 1988; Miyazaki 1991). The morphological identification of *T. callipaeda* has been reviewed by Otranto et al. (2003a). In both sexes, the mouth opening has a hexagonal profile. The internal margins of the buccal capsule are everted and subdivided by excavations into 6 festoons. SEM observations on the female *T. callipaeda* showed only one pocket-shaped amphid at the anterior end (WY Choi et al. 1989). On the mouth opening of male *T. callipaeda*, two large head papillae were observed, which were absent in the females and were distinct from the cephalic papillae in both their morphology and orientation (WY Choi et al. 1989). Adult female *T. callipaeda* measure 12–18.5 mm in length and 370–510 μ m in width. The vulva, which has a short flap, is located in the anterior region of the body, whereas the anal opening is 70–102 mm from the caudal end. The vagina opens at 62.0–162.2 mm (mean 108.7 $\pm$ 619.6 mm) anterior to the esophagus–intestinal junction. Two phasmids are present on the tip of the tail. The number of cuticular transverse striations was 400~650/mm on the head region, 250/mm on the middle region and 300~350/mm on the tail region. *T. callipaeda* is ovoviviparous rather than viviparous (Hong et al. 1988; Nagada 1964). Adult *T. callipaeda* males measure 7.7–12.8 mm in length and 338–428 mm in width at the mid-portion of the body. The caudal end is ventrally curved, without caudal alae. There are 15 pairs of papillae on the ventral surface of the caudal end, 10 of which are pre-cloacal and 5 post-cloacal. The first 3 of the 5 post-cloacal pairs are situated behind the cloaca near the anus, 1 pair in the middle, and another pair in the posterior-terminal position. The two dissimilar spicules are characterized by the well-defined shape of the anterior extremity of the longer left spicule and the typical crescent shape of the shorter right spicule (Otranto et al. 2003a).

T. californiensis can infect humans and cause ocular thelaziasis. The females are 12–18.8 mm long, with a vulva 800–1000 μ m from the anterior end. The eggs in vitro are 51 by 29 μ m, and are embryonated when laid. The males are 7.7–13 mm long, with unequal spicules 1.5–1.7 mm and 150–187 μ m long, respectively. A small gubernaculum may or may not present (Levine 1968). Also, 6–7 pairs of pre-cloacal papillae are seen at the posterior end of the male worm. *T. californiensis* is different morphologically from *T. callipaeda* based on the numbers of pre- and post-cloacal papillae in the male and the position of the vulva in the female (Bhaibulaya et al. 1970).

3.1.2 Life cycle

T. callipaeda was originally reported from Asian countries (Bhaibulaya et al. 1970; Shi et al. 1988), where human infections with this nematode are considered to be emerging over the last two decades, particularly in poor rural communities (JL Shen et al. 2006). *T. callipaeda* requires a vector which also acts as an intermediate host to accomplish its life cycle (Otranto et al. 2006a). Species of the dipteran family Drosophilidae (fruit flies, subfamily Steganinae) have been incriminated as vectors. *Amiota variegata*, recently taxonomically reclassified as *Phortica variegata* (Maca 1977), was first identified as the intermediate host and vector. However, *A. okadai* is also considered to be a vector of this parasite in China (summarized in Otranto et al. 2006a). *Phortica* spp. and, to a lesser extent, *Amiota* spp. display a zoophilic behavior, i.e. they feed on ocular secretions of animals and humans in addition to feeding

on fruits and on fermenting tree sap (Bachli et al. 2005). Interestingly, only males of *P. variegata* were found to be infected with *T. callipaeda* under natural conditions in Italy (Otranto et al. 2006a,b), whereas both male and female flies were positive in dissection and/or molecular assays under experimental conditions (Otranto et al. 2003c) (Fig. 1). An efficient method for trapping high numbers of such drosophilid flies under natural conditions and determining the presence and the population dynamics of *Phortica* and *Amiota* spp. in Switzerland was designed by Roggero et al. (2010). In this method, a large polyethylene terephthalate (PET) container was used, oriented horizontally, with a single aperture covered by a net (mesh 0.4 mm) and narrowing through a funnel towards the brighter side of the trap. The traps were installed close to each other (maximum distance 40 cm). All traps were baited with sliced fresh apples and peeled bananas, the bait being changed once per week, after collection of the flies. In another study which was carried out by Otranto et al. (2005a), the results clearly suggested that *Musca domestica* is not a vector of *T. callipaeda* under experimental or natural conditions. Also, local transmission of *T. callipaeda* in southern Germany was investigated by Magnis et al. in 2010. Transmission of *T. callipaeda* occurs when the intermediate host and vector *P. variegata* feeds on lachrymal secretions from an infected animal and/or human and ingests *T. callipaeda* first stage larvae (L1) produced by adult female nematodes in the conjunctival sac of the final host. First stage larvae penetrate the gut wall in a few hours. They remain in the abdominal haemocoel for about 2 days. On the third day, larvae invade the fat body of the female and the testes of the male, where they subsequently become encapsulate, grow and moult twice to third stage larvae ~ 14-21 days after infection. After migrating through the body cavity of the vector, the L3s of *T. callipaeda* emerge from the labella of infected flies, after which they feed on the lachrymal secretions from infected hosts and develop into the adult stage in the conjunctival sac and prebulbar tear film within ~ 35 days (Otranto et al. 2005c). The existence of a seasonal periodicity in the reproductive cycle of female nematodes, coinciding with the presence/absence of the vector/s, has been demonstrated by Otranto et al. (2004).

Fig. 1. *Phortica variegata*, female (Left), *Phortica variegata*, male (Right), (Courtesy Jorge Almeida, Viseu, Portugal).

Adult *T. californiensis* are commonly found in the tear ducts and conjunctival sacs of its hosts. Burnett et al. (1957) allowed laboratory-reared flies to feed on the sheathed larvae and discovered developmental stages in *Fannia canicularis* (Fig. 2). Similar larvae were found in wild-caught *F. benjamini* collected in an enzootic area in California.

Fig. 2. *Fannia canicularis*, female (Left), *Fannia canicularis*, male (Right) , (Courtesy Nikita E. Vikhrev, Moscow, Russia).

3.1.3 Epidemiology

T. callipaeda is prevalent in dogs, cats, and humans in the former Soviet Union and in countries of the Far East, including China, Korea, Myanmar, Japan, Indonesia, Thailand, Taiwan, and India. The parasite's distribution explains the use of the name, oriental eyeworm (Anderson 2000). However, the main final reservoir hosts of *T. callipaeda* seem to be farm dogs, as they often live in areas populated by a large hematophagous fauna. In an investigation carried out by Seo et al. (2002) in Korea, military dogs were found to be acting as reservoir hosts. Experimentally implanted adult worms of *T. callipaeda* have adapted to dogs, rabbits, cats and monkeys but not to goats and sheep, perhaps due to their different susceptibilities (Faust 1928).

The climate of the countries in which *T. callipaeda* has been reported varies from tropical (e.g. Indonesia) and subtropical (e.g. Japan) in the Far East, to temperate in the Russian Federation. In a study which was carried out in two distinct areas of Italy (the Piedmont region, North Western Italy and the Basilicata region, Southern Italy), dogs, cats and foxes were examined. *T. callipaeda* has been reported with high prevalence in dogs (60%), and foxes (49.3%) in southern Italy (Otranto et al. 2003b). The orography of the Basilicata region is characterized by the presence of a ring of sandstone mountains surrounding a woodlands bordering the study municipalities. These results confirm the hypothesis that the vectors of *T. callipaeda* need a mountainous environment for their survival and biological cycle, and the high prevalence in dogs and foxes indicates that *T. callipaeda* infection is hyper-endemic in this area (site B). Of the dogs examined from site A, 23.07% were found to be infected with eyeworms (Otranto et al. 2003b). Cats are less frequently in contact with the vector (i.e. dipteran flies), moreover it may be assumed that reports of thelaziosis from practitioners are rare due to difficulties in inspecting cats' eyes (Otranto et al. 2003b).

Canine thelaziasis has also been reported in France and Germany (Chermette et al. 2004; Hermosilla et al. 2004). In southern Switzerland (Ticino), the first case of *T. callipaeda* in a dog was detected in 2000 (Malacrida et al. 2008). *Thelazia* infection was also diagnosed in 5.6% of foxes shot in Ticino during the winter of 2005-2006. Affected foxes, dogs and cats originated from the same regions. The cats and 57.9% of the infected dogs had never crossed the Swiss border. In addition, five cats with thelaziasis were registered. Infected animals harbored 1-23 eye worms. The most common symptoms were conjunctivitis and epiphora, while keratitis was present only in a few animals. Young and small sized dogs were

significantly less involved than larger dogs over 3 years of age (Malacrida et al. 2008). There is little information on the role of wild carnivores as hosts of this nematode. Otranto et al. (2009) reported, for the first time, the infection of beech martens, wildcats, and brown hares by *T. callipaeda*. In addition, the retrieval of *T. callipaeda* in one of two wolves examined supported the previous report of nematodes in this animal species (Otranto et al. 2007). The finding of adult worms of *T. callipaeda* in the eyes of wildlife species indicates their competence as final hosts of this parasite, since it implies that third stage larvae, released by the intermediate host, developed to adults in their ocular cavity. From a parasitological viewpoint, these results are interesting because *T. callipaeda* has the broadest spectrum of final hosts among *Thelazia* species, while *T. lacrymalis* parasitizes only horses and *T. rhodesi* and *T. gulosa* mainly infect cattle (Anderson 2000).

The eyeworm, *T. californiensis* is confined to the Western United States (Dozie et al. 1996; Skrjabin et al. 1971; Soulsby 1965). It is a parasite of dogs and can infect cats, foxes, coyotes, sheep, deer, horses, rabbits and black bears. Humans can become infected, but it is extremely rare and thought to be accidental.

Both *T. callipaeda* and *T. californiensis* are responsible for human thelaziasis. The first report of human thelaziasis was from Beijing, China, where adult nematodes were removed from the eyes of a man (Stuckey 1917). In subsequent years, the number of human thelaziasis cases has increased in China (Chen and Zhang 1954), the Soviet Union (Miroshnichenko et al. 1988), Indonesia (Kosin et al. 1989), Thailand (Bhaibulaya et al. 1970; Yospaiboon et al. 1989), India (Singh and Singh 1993), Taiwan (Cheung et al. 1998; YJ Yang et al. 2006), Japan (Koyama et al., 2000), and Korea (Min et al. 2002; Youn 2009). Human thelaziasis occurs mostly in rural communities with poor living and socioeconomic standards, and mainly affects children and the elderly. It seems that the prevalence and relevance of human thelaziasis have increased in China in the last 2 decades. By October 2005, a total of 371 human cases were reported from different provinces in China (JL Shen et al. 2006). Epidemiological investigations of 179 human cases in China revealed that 51% of cases were females and 49% of cases were males; the majority of individuals were children less than 6 years of age (64.2%) (Jiang et al. 1991). The remainder was comprised of young people between 7 and 18 years of age (7.8%) and adults of >19 (19.0%) years of age; host age was not reported for 9.0% of the samples. The ocular infection was unilateral in 88.1% of patients. Furthermore, the parasites were detected in the anterior eye chamber in 4 patients from the provinces of Anhui, Jiangsu, and Guangxi, and in the vitreous body and on the retina in 1 patient from Sichuan province (CC Chen and Zhang 1954; YJ Wang et al. 2002). In China, cases of human thelaziasis have been reported mainly in rural areas (ZX Wang et al. 2002a), particularly where domestic dogs and other animals, for example, cats and foxes, are heavily infected (ZX Wang et al. 2002b; ZX Wang et al. 2003). For instance, the prevalence of thelaziosis in dogs in Guanghua county, Hubei province, was 95%, with as many as 52 nematodes recovered from a single dog. In Anhui province, more than 190 nematodes were isolated from a single dog (ZX Wang et al. 1998). Recently, human thelaziasis has been reported from China by W Chen (2010). Meanwhile, most clinical reports have been published in Chinese, Russian or Korean and have not been accessible to a large part of the international scientific community (JL Shen et al. 2006).

In 2008, Otranto and Dutto reported human infection by *T. callipaeda* in Italy and France in the same area where canine thelaziasis had already been reported. From a clinical viewpoint, these infections indicate the importance of this parasitic disease in differential

diagnoses from bacterial or allergic conjunctivitis. All cases of human thelaziasis were reported during the summer months, which is the period of *T. callipaeda* vector activity (Otranto et al. 2006a,b). Also, concurrence of human thelaziasis and allergic conjunctivitis (e.g. caused by pollens) in the spring and summer seasons may impair correct etiologic diagnosis of this disease (Otranto and Dutto 2008).

3.1.4 Pathogenesis and clinical signs

Human infections with *T. callipaeda* have received little attention, despite the high prevalence recorded in some Asiatic countries (reviewed in JL Shen et al. 2006), and human infections by *T. californiensis* have only been reported occasionally in the USA (Doezie et al. 1996). To our knowledge, with the exception of Japan, 157 cases have been reported worldwide (China, 124; Korea, 24; Thailand, 5; India, 2; Russia and Indonesia, 1 each). In Japan, approximately 100 cases have been reported, mostly (66 cases) in western regions, and especially in Kyushu (Koyama et al. 2000).

One human case, reported by Howard (1927), in which larvae of *T. callipaeda*, in an advanced stage of development, was found in a papilloma of the skin of the lower right eyelid near the internal canthus, may possibly have a bearing on the early stage of the mature infective larvae of this species present in mammalian infections. The patient, from whom the papilloma was excised, gave a history of having played with a pet dog which had an irritation of both eyes, although "eye worms" were not actually observed in the dog. There was no evidence that the patient himself had ever been parasitized by adult *Thelazia*. The papilloma, which had been present for many years, developed a persistent itching several months prior to Dr. Howard's examination of the case. The patient was conscious of having frequently rubbed the wart during the period when the dog was being played with. Scratching resulted in the development of an indurated scab-like encrustation around the lesion. Microscopic slides of the excised papilloma, showing the worms in position in the tissue, were referred for diagnosis. The nematodes were directed head-inward into pockets produced by the infolding of the epithelium, or had migrated into the ducts of the sebaceous glands. In another case, the epithelial folds were strongly cornified and the tissue just beneath the epithelium was characterized by a marked lymphocytic infiltration. Two or more worms were found in each focus (Faust 1928; Faust et al. 1975).

In an experimental infection conducted by ZX Wang and Yang (1985) the clinical signs induced by thelaziasis were studied and it was demonstrated that adult parasites transplanted into the eyes of rabbits induced an inflammatory reaction within 3–5 hr. Swelling of the eyelids, congestion of the conjunctiva, and yellow secretions from affected eyes indicated a purulent inflammation; the extent of symptoms gradually decreased after 1 wk of infection. The results of another study showed that heavy experimental infection (with 40–50 worms) led to blindness in 3 of 20 rabbits, as a consequence of leukoma (ZX Wang et al. 2002b; ZX Wang et al. 2006).

The most common clinical manifestations in natural infections are mild conjunctivitis, follicular hypertrophy of the conjunctiva, excessive lachrimation, ocular secretions, itchiness, congestion, swelling, hypersensitivity to light, and keratitis, depending on the number of nematodes present in the eye, their location, and the host response (ZX Wang and Yang, 1985; KC Wang et al. 1999). In some cases, patients show redness of the eye, discharge, photopsia, decreased vision and "floaters" within the eye chamber, a partially blocked field of vision, or even complete vision loss (Zakir et al. 1999). Follicles, ulcers,

nubecula, swelling, paralysis of the eye muscles, ectropion, and papilloma, have also been reported in some patients (Ohira 2000). In addition, the presence of the nematodes causes infants to rub their eyes, and symptoms become more serious upon secondary infection with bacteria (*Pasteurella*, *Chlamydia*, and/or *Staphylococcus*). Localization to the anterior chamber, the vitreous body, or the retina are often associated with the development of fibrous tissue, inducing more serious clinical manifestations, for example, black spots in the visual field, ocular congestion, and sometimes purulent exudation under the anterior chamber (CC Chen and Zhang, 1954; YJ Wang et al. 2002). Compared with the eye ball of the host, the adult worm is a fairly large, live foreign body. It is generally noticed at an early stage and usually examination of the patient reveals the adult and/or larval nematodes (Fig. 3), mostly in the conjunctival sac or medial or lateral canthus of the eye (JL Shen et al. 2006).

Fig. 3. Conjunctivitis in a human patient infected by adult *Thelazia callipaeda* (Courtesy Domenico Otranto, Bari, Italy).

In Taiwan, a 62-year-old woman presented with unilateral eye swelling and itching (YJ Yang et al. 2006). Subsequent examination showed *T. callipaeda* infection. Although this is a known form of ocular infection, especially in Asia, this is one of few reported cases in Taiwan.

In a study from Italy, a case of ocular thelaziasis was reported in a man living in Piedmont (northern Italy). The patient first complained of hyperlacrimation and conjunctivitis. After a series of treatments with eye drops, the nematode responsible for the symptomatology was identified (Dutto 2008). These parasites are rather rare in Italy especially in the north of the country. Otranto and Dutto (2008) reported *T. callipaeda* infection in 4 human patients in Italy and France in the same area where canine thelaziasis had been reported. The 4 male patients (37–65 years of age) lived in northwestern Italy and southeastern France, where infections had been reported in dogs, cats, and foxes (Dorchies et al. 2007; Rossi and Bertaglia 1989). All patients showed exudative conjunctivitis, lacrimation, and foreign body sensation for a few days to weeks before referral.

In 2010, Kim et al. reported a case of intraocular infestation with *T. callipaeda* in a patient who was successfully treated by a pars plana vitrectomy in South Korea. A 74-year-old woman came to the hospital complaining of visual disturbance and floaters in the left eye. She had redness but no pain. A white, thread-like, live and mobile worm was observed in the vitreous cavity, and identified as an adult male *T. callipaeda* (Fig. 4). In 2006, the left eye of a 50-year-old man from Seoul, Korea was injured by shattered glass from the broken lens of his spectacles. Clinical examination of the eye revealed a 6mm-sized peripherally located,

single, full thickness corneal laceration with iris incarceration and traumatic lens opacity. The corneal wound was repaired. Two days after the operation, a 15 mm live thread-like worm was found inside the vitreous cavity. Trans pars plana vitrectomy for removal of the worm was performed, and the worm was identified as *T. callipaeda* (Lee et al., 2006). There have been only a few case reports about intraocular infestation by *T. callipaeda*. In these reports, all of the parasites were found in the anterior chamber except in one case where the worm was found in the vitreous cavity (Mukherjee et al. 1978; Zakir et al. 1999).

Fig. 4. A live *Thelazia callipaeda* worm in the vitreous cavity was clearly observed (Courtesy Hyun Woong Kim, South Korea).

Recently, the first case of human thelaziasis was reported from Bangladesh (Hossain et al. 2011). A 58 year old man was admitted with itching, redness, foreign body sensation, lacrimation and filamentary discharge from the right eye. He had conjunctival congestion and advanced bi-headed pterygium of the right eye. After exposure of the right eye ball with a universal eye speculum, a thin white nematode was found in the right lower conjunctival fornix. The nematode was identified as an adult *T. callipaeda*. In a previous report, small, white, thread-like, motile worms were recovered from the conjunctival sac of a 13-year-old girl and a 50-year-old woman from Dibrugarh district, Assam, India. They were identified as *thelazia* species (Nath et al., 2008). In another investigation, *T. callipaeda* was isolated from the conjunctival sac of a 32 year-old woman residing in the Himalaya Mountains (Sharma et al., 2006). Also, more intraocular infestations with *T. callipaeda* were reported from China (W Chen et al. 2010; Leiper 1917; Lv et al., 2009; Xue et al. China 2007).

T. callipaeda infects dogs, cats, foxes, rabbits (Kozlov 1962; Skrjabin et al. 1971), and wolves (Otranto et al. 2007). In affected wild animals, *T. callipaeda* adult and larval stages (Fig. 5) may cause mild ocular manifestations (conjunctivitis, epiphora, and ocular discharge) to severe disease (keratitis and corneal ulcers) (Otranto and Traversa 2005). In dogs and cats, however, chronic conjunctivitis will usually result, with manifestations such as photophobia and sometimes accompanied by blepharitis marginalis, lacrimation, corneal opacity, ulceration of the cornea, or even corneal perforation. The most common symptoms in affected dogs and foxes in Switzerland were conjunctivitis and epiphora, while keratitis was present only in a low number of animals. Young and small sized dogs were significantly less involved than large animals over 3 years of age (Dorchies et al. 2007; Malacrida et al. 2008).

Fig. 5. Both adult and larval stages of eyeworms are responsible for eye disease with symptoms ranging from mild e.g. lacrimation, ocular discharge, epiphora (Left) to severe e.g. conjunctivitis, keratitis and, corneal opacity or ulcers (Right) (Courtesy Domenico Otranto, Bari, Italy).

In humans, *T. californiensis* worms tend to reside in the superior and inferior fornices of the eye (Fig. 6). The most common clinical findings in infected patients include a mild conjunctival inflammation, foreign body sensation, follicular hypertrophy of the conjunctiva and possibly excessive lacrimation. The worms may occasionally migrate across the ocular surface, eventually causing corneal scarring, opacity and blindness. Human infection is extremely rare and thought to be accidental (Knierim and Jack 1975; Krischner et al. 1990).

Fig. 6. Adult specimens of *Thelazia californiensis* on the eye of a man (Courtesy Yan Peng, Dallas, USA).

T. californiensis was found on the conjunctivas of six of seven deer from Zion National Park, Utah. Based on field observations, adults appeared to be affected clinically at a higher incidence during both years of the study (1992-1994) as opposed to juveniles. Corneal opacity was the most apparent clinical sign from 1992 to 1993. However, in the following year, blepharospasm and epiphora were noted often (Knierim and Jack 1975; Taylor et al. 1996). Also, *T. californiensis* was found in 15% of hunter-harvested deer in Utah in 1994 and in 8% in 1995. Three live animals showed clinical signs of infectious keratoconjunctivitis (IKC) in 1996, but pathogenic bacteria were not isolated from these individuals (Dubay et al. 2000).

3.1.5 Diagnosis

The clinical diagnosis of thelaziosis in animals and humans may be difficult if only small numbers of nematodes are present, because clinical signs relate to an inflammatory response linked predominantly to the presence of developing third-stage larvae (L3) and/or fourth-stage larvae (L4), similar to an allergic conjunctivitis (Otranto and Dutto 2008).

A definitive diagnosis is made by the detection of the parasites in the conjunctival sac. Examination of lacrimal secretions may reveal eggs or first-stage larvae. However, morphological differentiation between *T. callipaeda* and *T. californiensis* is based on the numbers of pre- and post-cloacal papillae in the male and the position of the vulva in the female (Bhaibulaya et al. 1970; WY Choi et al. 1989; Miyazaki 1991; Otranto et al. 2003a). Also, in a study which was carried out on molecular characterization of *Thelazia callipaeda* (Otranto et al. 2005b), the results for the SSCP (single-strand conformation polymorphism) analysis and sequencing were concordant, indicating that the mutation scanning approach provides a useful tool for investigating the population genetics and molecular ecology of *T. callipaeda*.

3.1.6 Treatment, prevention and control

Due to the localization of the nematode, thelaziasis can be treated topically by direct application of drugs into the eyes. Mechanical removal of the adult parasites with fine forceps or cotton swabs using local anesthesia, is helpful. The clinical signs, excluding secondary infections with other pathogens, usually resolve rapidly after the removal of the worms (KC Wang et al. 1999). Patients with an intraocular infestation with *T. callipaeda* had a successful recovery after a pars plana vitrectomy. Untimely or incorrect treatment of the infection may result in a delay in recovery, mainly in children and the elderly, who are most likely to be exposed to infection by the fly. Thus, prevention of human thelaziasis should include control of the fly vector by use of bed nets to protect children while they are sleeping and by keeping their faces and eyes clean. Genetic identification of haplotype 1 has shown that this is the only haplotype circulating in animals (dogs, cats, and foxes) in Europe (Otranto et al. 2005b). This finding confirms the metazoonotic potential of *Thelazia* spp. infection and the need to treat infected domestic animals, which may act as reservoirs for human infection (Otranto and Dutto 2008).

The treatment for canine infection with *T. callipaeda* is to remove the worm. In the case of dogs having numerous worms, they must be eliminated under general anesthesia by turning up the third eyelid. Michalski (1976) found 2 ml of levamisole injected into the subconjunctival sac was more effective than levamisole given orally. Also, application of organophosphates (Rossi and Peruccio 1989) or 1% moxidectin (Lia et al. 2004) is effective. Meanwhile, the treatment of dog thelaziasis caused by *T. callipaeda* using a topical formulation of 10% imidacloprid and 2.5% moxidectin was studied by Bianciardi and Otranto in 2005. A clinical trial of ivermectin against eyeworm in German Shepherd military working dogs carried out by Fudge et al. (2007). Although ivermectin does not prevent dogs from being infected with eyeworms, the study suggests that ivermectin administered orally at a dose of 0.2 mg/kg every 3 weeks significantly reduces the prevalence of *Thelazia* species eyeworms in dogs. The efficacy of the prophylactic use of a monthly treatment with milbemyacin oxime showed 90% efficacy against *T. callipaeda* in naturally exposed dogs (Ferroglio et al. 2008).

The treatment of human *T. californiensis* conjunctival infestation is fairly straight forward. The symptoms usually resolve immediately after removal of the worms. Therefore, *T. californiensis* infestation should be included in the differential diagnosis of patients with chronic conjunctivitis following hiking or camping in the mountains or back-country. Irrigation with Lugol's iodine or 2-3% boric acid is recommended immediately after worm removal or for parasites that are in the lacrimal ducts where they cannot be removed manually. Levamisole, either orally or parenterally, at 5mg/kg or 2ml injected into the conjunctival sac has also been recommended. A dose of 1mg/lb of Ivermectin given subcutaneously has been shown to cure similar infestations in Asia and Europe. There is no vaccine for thelaziasis (Doezie et al. 1996; Kirschener et al. 1990; Peng et al. 2001).

4. Animal thelaziasis

As *T. callipaeda* and *T. californiensis* infect both humans and animals, they were already discussed in the section of human thelaziasis. Thus, in the following section, the other *Thelazia* species, which only infect animals, will be discussed.

4.1 *Thelazia rhodesi* Desmarest, 1822

T. rhodesi occurs on the surface of the cornea, under the nictitating membrane, in the conjunctival sac and in the lachrymal duct of cattle, buffalo, zebu, bison, and less commonly horses, sheep and goats.

The body is milky-white, with thick, prominent transverse striations. The cephalic region is similar in both sexes. The stoma is short and broad, widest at the middle, and has no lips. Around the mouth, four pairs of sub-median small, conoidal cephalic papillae and two lateral amphidal apertures are seen (Fig. 7, Top, Left). Nematode amphids exist in a variety of forms and sizes, some probably purely chemosensory, some photoreceptive, with an associated gland (McLaren 1974). Also, there are two lateral cervical papillae, one on each side, 350–384 μ m from the anterior end (Fig. 7, Top, Right). These organs determine whether the nematode can pass through a small space (McLaren 1976). At the anterior end of both sexes, an excretory pore is seen (Naem 2007a).

The females are 12.5–20.5 mm long and 300–500 μ m wide. At the anterior end of the body, the vulva is located in the esophageal region, 505.2–536.3 μ m from the cephalic region. The pattern of the cuticle around the vulva is different from the rest of the body (Fig. 7, Bottom). At the posterior end, the anal pore is seen, and the tail end is stumpy with two phasmids near its extremity (Naem 2007a). The eggs are 26–29 μ long at first, but later when stretched by developing larvae they are 207 by 4 μ (Levine 1968). The males are 7.5–14.5 mm long and 420–475 μ m wide. The tail is blunt, and without caudal alae, with dissimilar and unequal spicules 624–850 μ and 100–130 μ long, respectively, and no gubernaculum. There are 14 paired pre-anal papillae, one single papilla directly anterior to the cloaca, one paired post-anal papilla, and two nipple shaped phasmids at the posterior end (Naem 2007a).

The females are viviparous. The first-stage larvae are passed by females into the lachrymal secretions where they are ingested by non-biting Diptera flies. It has been confirmed that *Musca autumnalis* and *Musca larvipara* are suitable intermediate hosts (Anderson 2000; Giangaspero et al. 2004). The first stage of *Thelazia* is very short-lived in the lachrymal secretion, only surviving a few hours, and transmission depends upon the continuous presence of the vectors. For this reason, thelaziasis has a seasonal occurrence according to the seasonality of the intermediate hosts (Dunn 1978).

Fig. 7. SEM of adult female's mouth, *Thelazia rhodesi* (Top, Left); SEM of cuticular sensory papillae of *T. rhodesi* (Top, Right); SEM of adult female's vulva, *T. rhodesi* (Bottom), (Courtesy Soraya Naem, Urmia, Iran), "With kind permission of Springer Science+Business Media."

T. rhodesi is particularly common in the Old World. Bovine thelaziasis was first reported in Iran by Ebadi in 1951. Other investigations indicate the presence of bovine thelaziasis in Japan, Ghana, Afghanistan, USA, Canada, UK, Italy, and Zambia (Arbuckle and Khalil 1978; Barus et al. 1976; Genden and Stoffolano 1980; Ghirotti and Iliamupu 1989; Krafur and Church 1985; Okoshi and Kitano 1966; O'Hara and Kennedy 1991; Munangandu et al., 2011; Turfrey and Chandler 1978; Vohradsky 1970). In a study conducted at an abattoir located in Cagayan de Oro City, Philippines, 23% of examined animals were infected with *T. rhodesi*. In nearly half of the animals, both eyes were infected. *T. rhodesi* infection was significantly more common in cattle of more than 3 years of age (25%) than in younger animals (15%). Ocular lesions were observed in 73 (11%) of examined cattle (Van Aken et al. 1996). This eyeworm was also reported in southern regions in Italy and the ecology of *Thelazia* spp. in cattle and their vectors has been documented by Giangaspero et al. (2004). In another survey, change in the prevalence of *Thelazia* species in bovine eyes was studied in England (Tweedle et al., 2005).

The adult and larval stages live in eyes causing conjunctivitis, keratitis, lacrimation, ocular discharge, and ulcers (Ikeme 1967; Otranto and Traversa 2005).

A definitive diagnosis is made by the detection of the nematode in the conjunctival sac. Examination of the lacrimal secretions may reveal eggs or first stage larvae (Soulsby 1986). The morphological descriptions (Guttekova 1987; Kikuchi 1976) and molecular findings (Otranto et al. 2001; Otranto et al. 2003c; Otranto and Traversa 2004; Tarsitano et al. 2002) already discussed have demonstrated the differences among *Thelazia* species.

Removal of the adult nematodes with fine forceps, using local anesthesia is helpful. The use of "nilverm" (tetramisole) in the control of clinical signs of *T. rhodesi* infection in cattle has been studied by Aruo in 1974. Halpin and Kirkly (1962) have reported that methyridine produces rapid recovery from *Thelazia* infection, as do tetramisole and levamisole given orally or parenterally. Eye salves containing 4% morantel tartrate or 1% levamisole have also been used with success. A case of eye infection in a heifer was reported with bilateral blindness, cornea opacity, excessive lachrimation and nasal discharge. Treatment with 6-10 drops of a 10% solution of levamisole resulted in a complete recovery, with a total of 127 adult *T. rhodesi* being recovered from the eyes (Salifu et al. 1990). Also, ivermectin is effective against *T. rhodesi* in the eyes of cattle (Kennedy 1994; Soll et al. 1992).

4.2 *T. lacrymalis* Gurlt, 1831

T. lacrymalis occurs on the surface of the cornea and conjunctiva, under the nictitating membrane, in the lacrymal gland and its ducts and in the ducts of the third eyelid gland (Dongus et al. 2000; Giangaspero et al. 1999, 2000a; Lyons et al. 1981). It has been found occasionally in the aqueous humor of the eye. *T. lacrymalis* is a parasite of buffalo, camel, dog, and equine species, especially horses. It has also been reported from cattle (Levine 1968; Moolenbeek and Surgeoner 1980).

According to an SEM study, the body of the female was thin, whitish, attenuated at both ends, 12.5 mm long and up to 279 µm in width. The mouth was without lips and the buccal opening was round, and surrounded by four cephalic papillae and two amphids. There were two lateral cervical papillae, one on each side. On the ventral side of the anterior end of the body, the vulva was located 493 µm from the anterior end (Naem 2005). In a study carried out on *T. lacrymalis* obtained from a naturally infected horse, the vulva was situated 593 µm from the anterior end (Beelitz et al. 1997). The anal pore was located on the ventral side of the posterior end of the body, and there was one button-like sub-terminal phasmid on each lateral side of the female's tail (Naem 2005). These phasmids are involved in evaluating the intensity of a given stimulus and helping the worm to maintain itself in a suitable environment (McLaren, 1976). The males are 8-12 mm long, with spicules 170-190 and 130-140 µ long, respectively (Levine, 1968).

Thelazia spp. are generally described as being viviparous (Dunn 1978; Fiebiger 1947; Lohrer and Horning 1967; Tretjakowa 1960; Yamaguti 1961) or ovoviviparous (Anderson 1992). *T. lacrymalis* can be described as ovoviviparous (Craig and Davies 1937; Dongus et al. 2003; Ivashkin et al. 1979; Lyons et al. 1980; Skrjabin 1971). Transmission occurs by means of non-biting dipteran flies of the genus *Musca* (Muscidae) which feed on animal ocular secretions, tears and conjunctiva. The intermediate host in the USSR is *M. osiris* (Barker 1970). In the fly body, the parasite goes through further larval development (Anderson 2000; Ivashkin et al. 1979; Lyons et al. 1997; O'Hara and Kennedy 1991).

T. lacrymalis has so far been documented in Europe, Asia, North Africa, and North and South America (Anderson 1992; Barker 1970; Beelitz et al. 1997; Eckert 1992; Ladouceur and Kazacos 1981; Lohrer and Horning 1967; Lyons et al. 1986). In a survey, which was

performed on the occurrence of internal parasites in 461 horses (1-30 years old) slaughtered at the Linköping abattoir in central Sweden, 3.1% of examined horses were infected with *T. lacrymalis* (Hoglund et al. 1997). The results of another study indicated that *T. lacrymalis* has greatly increased in prevalence and intensity in central Kentucky (Dougherty and Knapp 1994; Lyons et al. 1976; Lyons et al. 1997).

Thelaziasis in horses is primarily a summer problem, comparable to the condition in cattle (Dunn 1969; Soulsby 1965). In a survey, which was carried out in Normandy, France, *T. lacrymalis* was recovered from 10.3% of examined horses (Collobert et al. 1995). Another survey evaluated the prevalence of *Thelazia* spp. in slaughtered native horses in the province of Bari, Italy (Giangaspero et al. 1999). Sixty horses (14.7%) were found parasitized by *T. lacrymalis*. This is the first report of *T. lacrymalis* infection in a horse in Italy (Giangaspero et al. 1999). In the following year, another similar survey was done in the Abruzzo region of Italy, where 39.06% of examined horses were found infected with *T. lacrymalis* (Giangaspero et al. 2000a).

The importance of *T. lacrymalis* in causing follicular conjunctivitis, ulcerative keratitis and ophthalmia has been documented (Giangaspero et al. 1999; Skrjabin et al. 1971). Occasionally the anterior chamber may be invaded, causing extensive endophthamitis and blindness (Grant et al. 1973; Skrjabin et al. 1971; Stewart 1940). Schebitz (1960) described a complicated case in an Egyptian horse which progressed from conjunctivitis to severe ulceration and granulation of the eye. However, in many cases, eyeworms have no pathogenic effect on the host, especially in larger animals (Soulsby 1986).

Diagnosis of the disease may be accomplished by irrigating the eyes with saline and examining centrifuged sediment for the first stage larvae. Schebitz (1960) also described finding larvae in the blood of infected horses and donkeys. *Post mortem* diagnosis may be accomplished by placing the lacrymal glands in warm saline, permitting larvae and adults to emerge (Skrjabin et al. 1971). Besides the potential implications for studies on vectors, a PCR-RFLP based assay on the first and/or second internal transcribed spacer (ITS1 and ITS2) of ribosomal DNA was developed for the detection of *T. lacrymalis* DNA in its putative vector/s (Traversa et al. 2005). Thus, it could be used for diagnosis of equine thelaziasis at the species level especially considering that horses can be occasionally infected by *T. skrjabini* (Lyons et al. 1976).

Treatment generally includes mechanical removal of worms under local anesthesia, irrigation of the eye with dilute solutions of topical antiseptics such as iodine or boric acid, and treatment of any corneal ulceration to suppress the development of opacity (Soulsby 1965). Activity of 15 compounds, given alone or in mixtures was evaluated in 102 equids. None of the compounds appeared to be active against *T. lacrymalis* (Lyons et al. 1981).

4.3 *T. gulosa* Railliet and Henry, 1910

T. gulosa is a parasite mainly of cattle, and occurs on the surface of the cornea, under the nictitating membrane, and in the conjunctival sac and lachrymal duct. The mouth is without lips, surrounded by four cephalic papillae and two amphids. There are two lateral cervical papillae, one on each side (Naem 2007b). Gibbons (1986) included one scanning electron micrograph of the anterior end of *T. gulosa* in her atlas. An electron microscopic study of the cuticle of two *T. gulosa* and *T. rhodesi*, carried out by Guttekova (1987), revealed very interesting architectonics of the body surface of these nematodes.

According to an SEM study, the body of the female was thin, whitish, attenuated at both ends, 8.0-11.5 mm long, and of 350-460 µm wide at the maximum body width. The vulva was located 460- 610 µm from the cephalic region. The anal pore was located 70-120 µm

from the posterior end of the body. There was one button-like sub-terminal phasmid on each lateral side of the female's tail (Naem 2007b). The males were 7.5–8.0 mm long and 300–370 μm wide at the maximum body width. The tail was blunt without caudal alae and curved ventrally. The number of pre-anal papillae was 35–40 (Fig. 8). These papillae were unpaired, and one single papilla directly anterior to the cloaca was also seen. Three pairs of post-anal papillae were present. The spicules were unequal (608–1025 μm and 120–125 μm long, respectively), dissimilar and showed a groove-like structure (Naem 2007b). There was no gubernaculum (Levine 1968).

Fig. 8. Coiled posterior pole of a *Thelazia gulosa* male, showing the 2 different spicula (Courtesy Soraya Naem, Urmia, Iran), "With kind permission of Springer Science+Business Media."

Like *T. lacrymalis* of horses, *T. gulosa* is now widely distributed in North America where it is transmitted by the face fly, *Musca autumnalis*, which was introduced to the continent in the early 1950s and has spread widely in the northern USA and the Canadian provinces (Chitwood and Stoffolano 1971; Chirico 1994; Greenberg 1971). Also, other *Musca* species act as intermediate hosts in the Ukraine, the Far East of CIS, the Crimea, and Europe (Branch et al. 1974; Krastin 1950a,b; Genden and Stoffolano 1981, 1982; Moolenbeek and Surgeoner 1980; Skrjabin et al. 1971; Vilagiova 1967). Kennedy and MacKinnon (1994) noted that the distribution of *T. gulosa* differed from that of *T. skrjabini* in the orbits of cattle. The two lachrymal ducts associated with the Harderian gland contained 58% of the *T. skrjabini* whereas the large ventral duct of the orbital glands contained 58% of the *T. gulosa*. The eyeworms were found more often in a lachrymal duct as a single species (90%) than as a mixed infection of two species (10%).

T. gulosa has been reported in the CIS, Asia, Australia, North America and Europe (Anderson 2000; Genden and Stoffolano 1980; Giangaspero et al. 2000 b; Gutierrez et al. 1980; Lyons and drudge 1975; Overend 1983; Patton and Marbury 1978). It occurs on rare occasions in England (Fitzsimmons 1966). *T. gulosa* found in cows in Poland and southeast Kazakhstan (Levine 1968). In another study, *T. gulosa* was reported in an imported giraffe in California and *T. lacrymalis* was identified in a native horse in Maryland (Walker and Becklund 1971). An eight month survey of bovine eyes from an abattoir in southern Ontario revealed the presence of *T. gulosa* and *T. lacrymalis* in 32% of the cattle (Moolenbeek and

Surgeoner 1980). In the aforementioned study (Moolenbeek and Surgeoner 1980), a total of 2,191 adult female face flies (*M. autumnalis*) were examined. Sixty-five (3%) of these harbored larvae of *Thelazia* spp. The prevalence of *Thelazia* spp. in cattle has been reported in southern Italy. *T. rhodesi*, *T. gulosa* and *T. skrjabini* were found in 80%, 34.5% and 1.8% of examined animals, respectively. This was the first report of *T. gulosa* and *T. skrjabini* in Southern Europe (Giangaspero et al. 2000b).

Oakley (1969) described a group of calves showing signs of lacrimation, keratitis and corneal opacity in which the presence of *T. gulosa* was demonstrated. Also, keratoconjunctivitis has been reviewed by Baptista (1979). Examination of histological sections of two heavily infected lacrimal ducts revealed evidence of chronic follicular conjunctivitis although this was considered not to be specific to *Thelazia* spp. (Moolenbeek and Surgeoner 1980).

Diagnosis of *T. gulosa* is made by the detection of the nematode in the conjunctival sac. Examination of the lacrimal secretions may reveal eggs or first stage larvae (Soulsby 1986). The morphological descriptions, and molecular findings (Guttekova 1987; Kikuchi 1976; Naem 2007b; Otranto et al. 2001; Tarsitano et al. 2002) demonstrate the differences among *T. gulosa*, *T. skrjabini*, and *T. rhodesi*.

The anthelmintic efficacy of doramectin was assessed for the control of *T. gulosa* and *T. skrjabini* in two studies using 44 naturally or experimentally infected calves (Kennedy and Phillips 1993). No eyeworms were found in any doramectin-treated animal. Also, the efficacy of ivermectin (pour-on) against the eyeworms *T. gulosa* and *T. skrjabini* in naturally infected cattle was studied by Kennedy et al. (1994).

4.4 *T. leesei* Railliet and Henry, 1910

T. leesei, is a parasite of the dromedary and the Bactrian camel in India, Europe (France), the USSR (Turkmenistan), and Africa (Kenya). The females are 14-21 mm long and 400 µm wide, with a vulva 425-440 µm from the anterior end. The males are 12 mm long and 210 µm wide, with unequal spicules 340 and 105 µm long, respectively (Anderson 2000; Aypak 2007; Levine 1968). *T. leesei* is transmitted by *M. lucidula* in Turkmenistan, and Infected flies were found around the eyes of dromedaries from the end of May to the beginning of October (Anderson 2000).

4.5 *T. alfortensis* Railliet and Henry, 1910

T. alfortensis occurs in the conjunctival sac of cattle in Europe, but is considered to be a synonym of *T. gulosa* (Soulsby 1986).

4.6 *T. skrjabini* Erschow, 1928

T. skrjabini is normally found within the lacrimal ducts of the third eyelid in cattle, and less frequently is found beneath the third eyelid or lacrimal ducts leading from the orbital lacrimal gland opening into the conjunctiva near the fornix. The cephalic region is similar in both sexes. The mouth is orbicular and has no lips with its anterior edge turned over and has six grooves. Around the mouth, two circles of cephalic papillae are seen: the inner circle with six papillae and the outer circle with four sub-median cephalic papillae. At both lateral sides of the head, two amphids are observed. The cuticle shows fine, scarcely visible transverse striations and two lateral cervical papillae (Naem 2007c).

The females are 11-19 mm long and 178-378 µm wide. The protruded vulva is located 410-710 µm from the cephalic region. The anal pore is located at the posterior end of the body

and the tail has two phasmids near the tip (Naem 2007c). The males are 5–9 mm long and 178–260 μm wide. The tail is blunt without caudal alae and curved ventrally. There are 31 to 38 unpaired pre-anal papillae, two paired post-anal papillae, and two nipple-shaped phasmids at the posterior end. The pattern of the cuticle around the cloaca is different from the rest of the body (Naem 2007c).

The mature females are viviparous. According to Krastin (1952) and Skrjabin et al. (1971), the intermediate hosts are *Musca amica* and *M. vitripennis*. O'Hara and Kennedy (1989,1991) orally inoculated *M. autumnalis* with first-stage larvae of *T. skrjabini*, which was recently introduced to North America.

T. skrjabini is common in cattle in Alberta, Canada (Kennedy et al., 1993) and is occasionally reported from other mammals including horses in the United States (Lyons et al. 1976), and buffalo in India (Bhopale et al. 1970; Pande et al. 1970). Also, this nematode occurs in cattle in, Denmark, Poland, USSR, and Australia (Anderson, 2000; De Chaneet 1970; Kolstrup 1974). Kennedy and Mackinnon studied site segregation of *T. skrjabini* and *T. gulosa* in the eyes of cattle (1994). Eyeworms occurred 90% of the time in a duct as a single species compared to 10% of the time as a mixed species infection. The data suggest that *T. skrjabini* and *T. gulosa* are more site specific than previously believed (Kennedy and Mackinnon 1994).

The adult and larval stages of *T. skrjabini* live in eyes, causing conjunctivitis, keratitis, lacrimation, and ocular discharge (Ikeme 1967; Otranto and Traversa 2005; Soulsby 1986). Diagnosis is made by the detection of the nematode in the conjunctival sac. Examination of the lacrimal secretions may reveal eggs or first stage larvae (Soulsby 1986). The morphological descriptions (Guttekova 1987; Kikuchi 1976; Naem 2007c) and molecular findings (Otranto et al. 2001; Otranto et al. 2003a; Tarsitano et al. 2002) indicate the differences among *Thelazia* species.

The efficacy of doramectin against *Thelazia* spp. in both naturally and experimentally infected calves was 100%, and no worms were found in any doramectin-treated animal (Kennedy and Phillips 1993; Marley et al. 1999). In another investigation, the efficacy of ivermectin was 97.02% and 100% against adults of *T. skrjabini* and *T. gulosa*, respectively (Kennedy et al. 1994).

4.7 *T. ershowi* Osterskaja, 1931

T. ershowi occurs in the tear ducts and the conjunctiva of pigs in the USSR. Osterskaja (1931) found the parasite in 7 of 350 pigs in the Ural area. The females are 5.0–8.7 mm long and 188–207 μm wide, with a tail 65–105 μm long and a vulva 395–489 μm from the anterior end. The males are 4.9–5.5 mm long and 176–188 μm wide, with a left spicule 152–155 μm long and a right spicule 114–115 μm long (Levine 1968).

4.8 *T. bubalis* Ramanujachari and Alwar, 1952

T. bubalis is a nematode of the conjunctival sac of the water buffalo in India. The females are 6.5–7.8 mm long and 250 μm wide, with a vulva 900 μm from the anterior end. The males are 6 mm long, with a left spicule 800 μm long and a right spicule 150 μm long (Levine 1968; Soulsby 1986).

4.9 *T. anolabiata* Molin, 1860

Twenty-two *Thelazia* species have been reported from wild birds (Anderson 2000). *T. anolabiata* occurs in the orbits of birds, which can cause lacrimation, keratitis, conjunctivitis,

and corneal ulcers. This species was reported for the first time from an Andean Cock-of-the-rock (*Rupicola peruviana*) from a zoo in Lima, Peru (Elias et al. 2008). The nematodes were identified as *T. anolabiata* based on the lengths of spicules (1770 μm) and other morphologic characteristics such as the number of anal papillae of the males (8 pairs of pre-anal papillae), and the appearance of the first annulations on the anterior part of the body (45 mm posterior to the buccal capsule). There is some controversy about the number of *Thelazia* spp. in South American birds. Although Anderson and Diaz-Ungria (1959) believed that both *T. anolabiata* and *T. digitata* were valid species, Rodrigues (1992) lists *T. digitata* and *T. lutzi* as synonyms of *T. anolabiata*. However, molecular assays would be of great use in validating the avian species of *Thelazia* (Elias et al. 2008). In the Peruvian study, clinical signs of the infected bird (keratoconjunctivitis) were resolved with treatment with ivermectin and ciprofloxacin. Other studies have documented avian thelaziasis in Israel, Senegal and Japan (Brooks et al. 1983; Murata and Asakawa 1999; Wertheim and Giladi 1977).

5. Conclusion

Among *Thelazia* species causing ocular infections in animals, *Thelazia callipaeda* and, more rarely, *Thelazia californiensis* have also been found in human eyes. Human and animal thelaziasis have been documented in Europe, Asia, North America, South America and South Africa. The adult nematode lives in the eyes and associated tissues, including under the lids and in the conjunctiva and lacrimal glands and ducts. The transmission of eyeworms occurs when flies acting as intermediate hosts, feed on lachrymal secretions from an infected animal and/or human and ingest first-stage larvae of *Thelazia* spp. produced by female nematodes in the conjunctival sac of the final host. The larvae develop into the third-stage larvae in the fly, and emerge from the labella of an infected fly, when it feeds on the ocular secretions of a new host. Larvae develop into the adult stage in the conjunctival sac within ~ 35 days. Disease caused by *Thelazia* spp. is characterized by a range of subclinical and clinical signs, such as lacrimation, epiphora, conjunctivitis, keratitis, and corneal ulcers. Diagnosis is made by the detection of eggs or first-stage larvae in lacrimal secretions or by finding the adult worms in the conjunctival sac. Species identification is confirmed on the basis of microscopic examination with reference to keys. Thelaziasis can be treated topically by direct application of drugs into the eyes, and removal of the adult parasites with fine forceps, using local anesthesia. An integration of medical and veterinary expertise is needed to improve scientific knowledge to control eyeworm disease.

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This book presents a number of interesting and useful aspects and facets concerning the clinical features, properties and therapeutical management of this condition. Dr. H. Mejía-López et al. present an interesting survey of the world-wide epidemiologic aspects of infectious conjunctivitis. Dr. U. Ubani evaluates conjunctival symptoms/signs participating in the clinical features of this disorder. Dr. A. Robles-Contreras et al. discuss immunologic aspects underlying possibly the conjunctivitis. Dr. Z. Pelikan presents the cytologic and concentration changes of some mediators and cytokines in the tears accompanying the secondary conjunctival response induced by the nasal challenge with allergen. Dr. S. Sahoo et al. summarize the treatment and pharmacologic control of particular clinical forms of conjunctivitis in general practice. Dr. S. Leonardi et al. explain the basic pharmacologic effects of leukotriene antagonists and their use for the treatment of allergic conjunctivitis. Dr. J.A. Capriotti et al. evaluate the therapeutical effects of various anti-adenoviral agents on the acute conjunctivitis caused by adenovirus. Dr. V. Vanzzini-Zago et al. assess the prophylactic use and efficacy of "povidone-iodium solution", prior the ocular surgery. Dr. F. Abazi et al. present the clinical features, diagnostic and therapeutical aspects of "neonatal conjunctivitis". Dr. I.A. Chaudhry et al. review the special sub-form of conjunctivitis, being a part of the "Trachoma". Dr. B. Kwiatkowska and Dr. M. Maślińska describe the clinical, pathophysiologic and immunologic features of conjunctivitis. Dr. S. Naem reviews the conjunctivitis form caused by *Thelazia* nematodes, occurring principally in animals.

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