



ELSEVIER

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar



Epidemiology of the eye worm *Thelazia callipaeda* in cats from southern Switzerland

B. Motta^{a,b,e}, F. Nägeli^b, C. Nägeli^b, F. Solari-Basano^c, B. Schiessl^d,
P. Deplazes^e, M. Schnyder^{e,*}

^a Veterinary Clinic, VetMoesa SA, via Signù, CH-6537 Grono, Switzerland

^b Veterinary Clinic, Via San Gottardo 128, CH-6828 Balerna, Switzerland

^c Arcoblu s.r.l., Via Milesi 5, I-20133 Milan, Italy

^d Novartis Animal Health Inc., Schwarzwaldallee 215, CH-4058 Basel, Switzerland

^e Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 266a, CH-8057 Zurich, Switzerland

ARTICLE INFO

Article history:

Received 28 December 2013

Received in revised form 21 March 2014

Accepted 5 April 2014

Keywords:

Thelazia callipaeda

Eye worm

Cats

Southern Switzerland

Risk factors

ABSTRACT

Thelazia callipaeda is a spiruroid nematode of dogs, cats and wild carnivores transmitted by zoophilic drosophilid *Phortica* flies and found in an increasing number of European countries. In cats the disease is diagnosed sporadically. This study presents an epidemiological investigation of feline thelaziosis, performed in southern Ticino, Switzerland, an endemic area for *T. callipaeda*. Between January 2009 and July 2011 2171 cats, having outdoor access and presenting for various reasons, were examined by in-depth eye examinations, and clinical and anamnestic data were collected. The overall prevalence of *T. callipaeda* in the study area was 0.8% (17/2171 cats, 95% confidence interval: 0.5–1.3%). Among cats showing ocular illness, the prevalence was 9.2% (11/120, CI: 4.7–15.8%). Cats with eye worms had no international travel history and were significantly more often diagnosed between June and December than during other months. With one exception, one single eye per cat was infested, each harboring between 1 and 10 eye worms (arithmetic mean: 2.8 per cat). One cat presented with conjunctivitis and ulcers, seven with conjunctivitis only and 3 with a mildly increased lacrimation, while 6 cats were asymptomatic. Significantly more male than female cats had eye worms and cats older than one year were overrepresented. No pure-bred cats were infested. This study confirms the establishment of this potentially zoonotic parasite in cats from the study area. Due to the clinical relevance and pain caused by the infestations, increased disease awareness and in depth eye examination for the detection of *T. callipaeda* in cats are recommended, even in absence of obvious clinical signs, in order to initiate appropriate anthelmintic treatment.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Thelazia callipaeda (Spirurida, Thelaziidae) is a whitish nematode of 0.5–1.8 cm length infesting the conjunctival

pouches and the lachrymal ducts of dogs, cats, rabbits, wild carnivores and humans (Anderson, 2000). Adult and pre-adult stages cause mechanical damage to the conjunctival and corneal epithelium (Otranto and Traversa, 2005), provoking lacrimation, conjunctivitis or even keratitis, uveitis and corneal ulcers (Bussi eras et al., 1996; Malacrida et al., 2008). Lacrimal secretions are of significance for the transmission of first stage larvae (L1) to the intermediate and

* Corresponding author. Tel.: +41 44 635 85 25; fax: +41 44 635 89 07.
E-mail address: manuela.schnyder@uzh.ch (M. Schnyder).

vector host, the *Phortica* flies (Diptera, Drosophilidae). Attracted by ocular discharge, the flies ingest L1 while feeding on conjunctival secretions of an infested animal and, after approximately 3 weeks, they also transmit the infective third stage larvae (L3) to a definitive host (Otranto et al., 2004). Without treatment, adult worms can persist up to one year behind the third eyelid.

The past and present occurrence of this parasite in humans in far Eastern countries spread from India (Singh and Singh, 1993) to Japan (Koyama et al., 2000; Shen et al., 2006), led to the common name of “oriental eye worm” for *T. callipaeda*. Its presence is meanwhile increasingly reported also in carnivores in Europe, starting from 1989 (Rossi and Bertaglia, 1989). High prevalence is reported in dogs from Basilicata, in southern Italy (Otranto et al., 2003, 2009), where the density of *Phortica* flies is high and their seasonal activity is consistent between May and October (Otranto et al., 2006). However, the presence of adequate intermediate vectors has also been detected in Switzerland in the northern part of the Alps, with a typical continental climate (Roggero et al., 2010). At present, *T. callipaeda* is focally endemic in several European countries, infesting domestic and wild carnivores in Italy (Otranto et al., 2009) and southern Switzerland (Malacrida et al., 2008), domestic animals in France (Dorchies et al., 2007; Ruytoor et al., 2010), and, recently, on the Iberian peninsula (Miro et al., 2011; Vieira et al., 2012). A single infested dog without travel history was diagnosed in Germany (Magnis et al., 2010).

In previous studies conducted between 2005 and 2007 in the southern part of Switzerland, in the Canton of Ticino, which borders northern Italy, a prevalence of 6.2% and 11.1% has been detected in dogs and foxes, respectively (Malacrida et al., 2008). Contemporaneously, only five cats were diagnosed with *T. callipaeda* in the same area. In a field efficacy study performed in Italy and Switzerland, a total of 31 cats with *T. callipaeda* were identified (Motta et al., 2012). Previous studies reported sporadic cases in five cats in Italy, three in France and two in Portugal (Di Sacco et al., 1995; Dorchies et al., 2007; Otranto et al., 2003; Rodrigues et al., 2012; Ruytoor et al., 2010; Soares et al., 2013).

The aim of this study was to investigate epidemiological data concerning feline thelaziosis from a region known to be endemic for *T. callipaeda* in dogs and foxes.

2. Materials and methods

2.1. Animals and study area

Between January 2009 and July 2011 a total of 2171 cats were presented to a veterinary practice, located in southern Ticino (Switzerland), for different health concerns. The criteria for the cats to be enrolled in this study were to be client-owned, at least 6 weeks old and weighing more than 0.5 kg body weight (BW). All cats were living around the Mendrisiotto region (101 km², latitude 45°52'N 8°59'E, altitude ranging from 277 to 571 m above sea level), an area bordering northern Italy. The climate is characterized by a mean monthly relative humidity (RH) varying between 60% and 75% and varying monthly precipitations between 52 and 196 mm (see climate diagrams

of Lugano and Stabio, www.meteoschweiz.admin.ch) and maximum monthly temperatures >20 °C between May and September. Around 60.6% of the Mendrisiotto territory is wooded, 19.2% is agricultural, 18.8% is urban and 1.4% is unproductive surface (Poretti Suckow et al., 2012).

2.2. Procedures

All cats were subjected to an in-depth ocular examination in order to detect *T. callipaeda* infestations. Cats subjected to surgery were anesthetised adopting a combination of xylazine (2–4 mg/kg BW, Xylazine[®], Streuli) and ketamine (S-Ketamin[®], 3 mg/kg BW, Dr. E. Graeub) administered intramuscularly, allowing the third eyelid to be raised by means of ocular tweezers for visualization of the nematodes. The conjunctival pouches of cats not subjected to surgery were inspected after the application of two drops per eye of a local anesthetic (oxybuprocaine, Novesin[®] 0.4%, Omnivision).

The number of (pre-)adult worms was counted in each eye: if there were less than 10 worms, the exact number was counted and the infestation intensity was recorded as mild (1–5 worms), moderate (6–10 worms) and severe (>10 worms). Eye lesions such as conjunctivitis, lacrimation, ulcers, keratitis and ocular discharge were also recorded and classified as mild, moderate and severe. Some of the owners of cats harboring *T. callipaeda* were extensively informed about the parasite in the context of a placebo controlled, blinded field study (Motta et al., 2012). These cats were followed for a period of 14 days through weekly examinations.

2.3. Statistical analysis

Data about gender, age and breed were examined and correlated to the infestation with *T. callipaeda*. For statistical analysis cats were divided by sex, age (cats younger than one year versus cats older than one year) and 2 breed groups (European short hair and crossbred cats versus purebred). The number and percentage of animals with ocular illness in each gender or age group were summarized in contingency tables and Fisher's Exact tests were applied for the statistical comparison between those groups, with the level of significance being $p < 0.05$. Tests were performed two-tailed. Fisher's Exact tests were performed with SAS[®] (SAS Institute Inc., Cary, NC, USA). Exact binomial 95% confidence intervals (CI) for means of binomial variables were calculated with unweighted data according to the method of Clopper and Pearson (1934). Graphics were performed using GraphPad Prism version 4.02 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

3. Results

3.1. Anamnestic data and reasons for consultation

All examined cats had access to both outdoors and indoors, were of either sex ($n = 1129$, 52% males, and $n = 1042$, 48% females) and predominantly European short hair (see Table 1). Reasons for presenting at vet practices

Table 1

Sex, age and health concerns of 2171 cats from the Mendrisiotto region (southern Switzerland) inspected for infestation with the eye worm *Thelazia callipaeda*.

	All examined cats (n=2171)			Cats with ocular illness (n=120)			Cats with <i>T. callipaeda</i> infestation (n=17)		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
Sex									
Male	1129	52.0	49.9–54.1	60	50	40.7–59.3	13	76.5	50.1–93.2
Female	1042	48.0	45.9–50.1	60	50	40.7–59.3	4	23.5	6.8–49.9
Age ^a									
<1 year	302	13.9	12.5–15.4	20	16.7	40.5–24.6	1	5.9	0.1–28.7
>1 year	111	5.1	4.2–6.1	100	83.3	75.4–89.5	16	94.1	71.3–99.9
Unknown	1758	81.0	79.2–82.6	n.a.	n.a.		n.a.	n.a.	
Breed									
ESH ^b /crossbred				106	88.3	81.2–93.5	17	100	83.8–100.0
Purebred				14 ^c	11.7	6.5–18.8	0	0	0–16.2
Health concerns									
Ocular illness	120	5.6	4.6–6.6	n.a.	n.a.	n.a.	8	47.1	23.0–72.2
Vaccination	1004	46.2	44.1–48.4	n.a.	n.a.	n.a.	0	0	0–16.2
Spaying	130	6.0	5.0–7.0	n.a.	n.a.	n.a.	0	0	0–16.2
Neutering	153	7.0	6.0–8.2	n.a.	n.a.	n.a.	0	0	0–16.2
Other	764	35.2	33.2–37.2	n.a.	n.a.	n.a.	9	52.9	27.8–77.0

^a For more details on the age of cats with ocular illness and with *T. callipaeda* infestation see Fig. 2.

^b ESH, European short hair.

^c 7 Persian cats (2 neutered males, 3 neutered and 2 intact females); 3 Main Coon cats (all neutered males), 2 Ragdoll cats (1 neutered male and female respectively), one Sorian and a British Shorthair cat, both neutered males.

were vaccinations (1004), neutering (153), spaying (130) and specific consultations due to ocular problems (120). These included *T. callipaeda* affected cats with clinical signs (see Fig. 1). Other reasons included medical reasons (764) such as diarrhea, vomiting and apathy.

3.2. Cats with ocular illness

Cats with ocular illness were classified as follows: 80% (n=96) showed conjunctivitis, 17.5% (n=21) had ulcers, and 0.8% (one cat) had combined ulcer and conjunctivitis, while 1.7% (n=2 cats) harbored *T. callipaeda* and showed very mild lacrimation detected at veterinary examination but unnoticed by their owners. There was no difference

between the number of males and females affected, whilst in the age categories, differences were significant ($p=0.002$), with animals younger than one year being less often presented for ocular illness. Fourteen cats with ocular illness were purebred (of which, 7 were Persians, see Table 1, note 3); none of them were harboring *T. callipaeda*. Among the animals with ocular illness, 9.2% (11/120, 95% CI: 4.7–15.8) were *T. callipaeda* positive.

3.3. Cats with *T. callipaeda*

Overall prevalence of feline thelaziosis was 0.8% (17/2171, 95% CI: 0.5–1.3%). Among the cats with *T. callipaeda* infestation, only 11 showed ocular clinical signs.

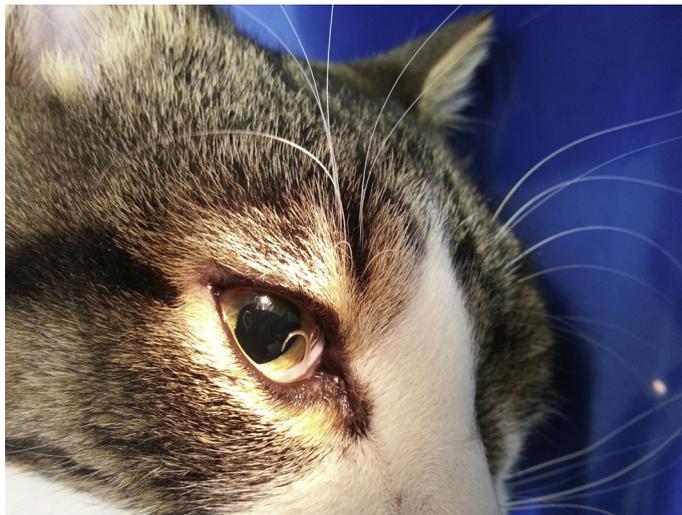


Fig. 1. *Thelazia callipaeda* in the eye of a cat from the Mendrisiotto region (Ticino, southern Switzerland) showing increased lacrimation.

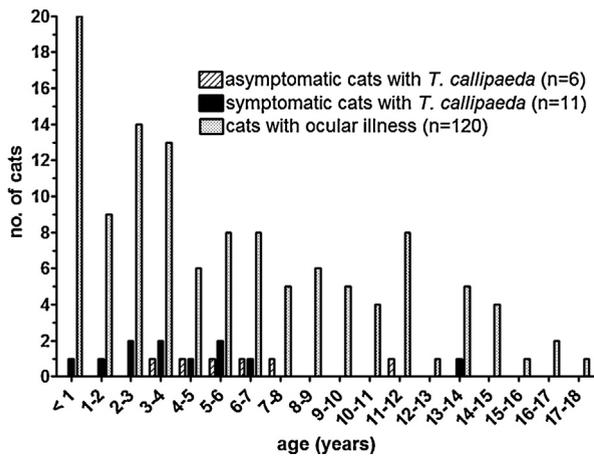


Fig. 2. Age of 120 cats examined for ocular concerns (dotted column) and of 17 cats harboring *Thelazia callipaeda* examined: 6 were asymptomatic (striped column) and 11 showed clinical signs (black column). Cats were visited between January 2009 and July 2011 in a veterinary practice in the Mendrisiotto region (southern Ticino, Switzerland).

Nine cats had conjunctivitis, of which one single cat also had ulcers in the same eye, the same cat as mentioned above; this neutered male, 8 year old, crossbred Norwegian cat harbored more than 10 *Thelazia* specimens. Two further already mentioned cats manifested mild lacrimation. A total of 9 of the *Thelazia* positive cats were presented by the animal owner for problems not connected with ophthalmic diseases. One cat was visited due to anorexia and fever, another due to cystitis, two cats due to abscesses, three for a health check-up and another one due to infection of the upper respiratory tract. This latter cat, together with a further cat from the region of Morbio Inferiore, was checked due to living with a *Thelazia* affected cat and dog respectively.

In contrast to the cats with ocular illness, where the ratio was balanced, significantly more male than female cats ($p=0.029$) were infested with *T. callipaeda* (Table 1). One single animal (male cat) was intact, while the others were neutered. Furthermore, significantly more cats infested with *T. callipaeda* were older than one year (16/17 cats, 91.1%, CI: 71.3–99.9). More precisely, one single *Thelazia* positive cat was aged 8 months, two cats were older than 11 years, while the remaining 14 cats were aged between 2 and 7 years (Fig. 2). In contrast, the age of cats with ocular illness varied from cats younger than one year (20 out of 120, 16.7%) up to 18 years, with a peak in the former ones and a logarithmic decreasing trend ($R^2=0.8016$) with increasing age (Fig. 2). Significantly less purebred cats with ocular illness were examined, and no purebred cat was diagnosed with *T. callipaeda* infestation.

The number of *Thelazia* specimens per eye varied from 1 to more than 10 (arithmetic mean: 2.8 worms per eye). Most cats harbored 1–2 eye worms, only 2 cats had an infestation with 4 and 5 *Thelazia* specimens per eye, respectively, and in the single cat harboring more than 10 eye worms in the same eye the exact number was not determined (10 was used for calculations). Ten cats had ocular worms in the right eye, while 6 of them in the left eye. One single intact female cat had *Thelazia* specimens in both

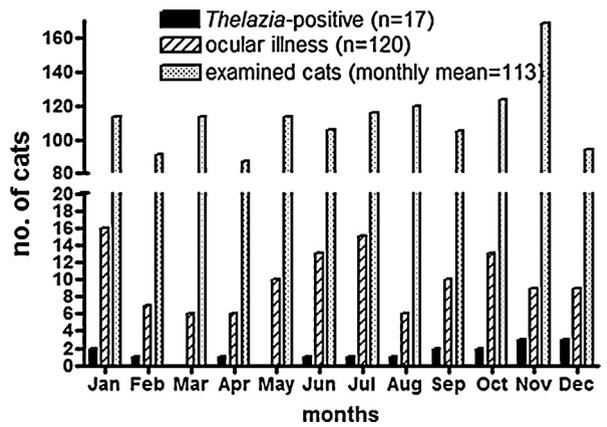


Fig. 3. Seasonal distribution of *Thelazia callipaeda* positive cats (black column) and of cats examined for other ocular problems (striped column) between January 2009 and July 2011 in a veterinary practice in the Mendrisiotto region (southern Ticino, Switzerland). These columns are itemized with the mean number of visited cats for each month during the same period in the same veterinary practice (dotted column).

conjunctival pouches (2 worms in the right and 1 in the left eye).

None of the *Thelazia* affected cats had a history of travel to Italy or to other countries known to be endemic for *T. callipaeda*. Four cats were diagnosed during winter and spring (23.5%, 95% CI: 6.8–49.9) between January and May, while the other 13 cats were diagnosed between June and December (76.5%, 95% CI: 50.1–93.2), indicating a significant difference. This is in opposition to cats with ocular illness, which were diagnosed throughout the year, with highest numbers (16 cats) found in January (Fig. 3).

The geographical distribution of the cats with ocular illness is shown in Fig. 4. Cases are clustered in and around centers and residential areas, in correlation with the human, and therefore also the cat population. For a clearer presentation, 2 cases with ocular illness from northern Ticino are not shown. These 2 cats were both neutered males and one of them was *T. callipaeda* positive.

4. Discussion

Here we present epidemiological data about feline thelaziosis in a region known to be endemic for dogs and foxes. The occurrence of *T. callipaeda* in cats is expected in particular in regions with high prevalence in dogs or foxes, as confirmed while recruiting cats for field efficacy studies in Italy and Switzerland, where 20 and 11 cats respectively were diagnosed (Motta et al., 2012). The prevalence of 0.8% (95% CI: 0.5–1.3%) in cats as determined by this study appears to be low. A previous investigation performed in the same area evidenced a prevalence of 6.2% and 11.1% in dogs and foxes, respectively (Malacrida et al., 2008), while 5 cats were diagnosed between 2000 and 2007, presenting with conjunctivitis (2 cats) and keratitis (1 cat). The 17 positive cats of this study were diagnosed within a much shorter time. However, 9 of them were brought to the veterinarian for reasons not related to ocular problems and among these, 6 cats were asymptomatic. In these 9 cats, the infestation with *T. callipaeda* could have been missed out of

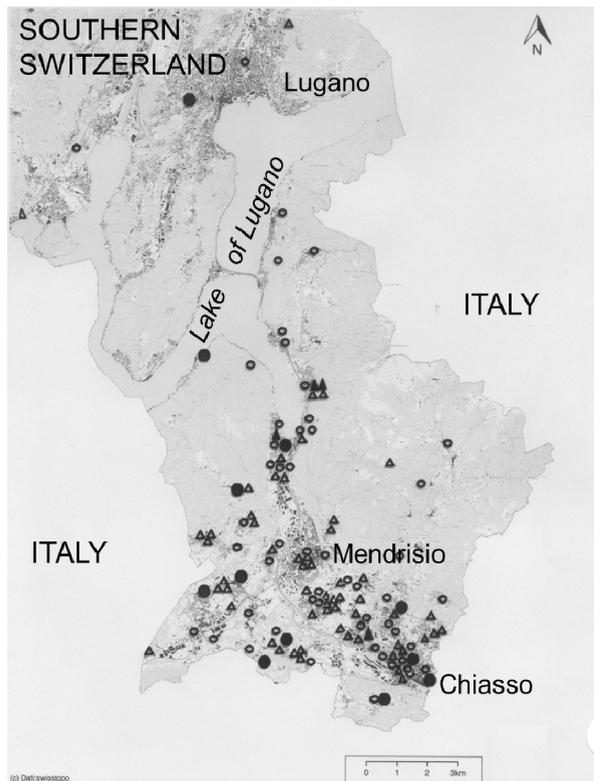


Fig. 4. Localization of 124 cats (60 females = triangles, 64 males = circles) visited between January 2009 and July 2011 in a veterinary practice in the Mendrisiotto region (southern Ticino, Switzerland). Symbols for 17 cats infested with the eye worm *Thelazia callipaeda* are filled in: 11 were showing ocular illness, 6 were asymptomatic. All other cats were presented for ocular illness not related to *T. callipaeda* infestation.

the context of this study. Cats generally showed poor or no clinical signs, confirming that such infestations may easily pass unobserved by cat owners, and also by veterinarians: in-depth eye inspection in cats is not a routine analysis for practitioners because cats need to be well sedated or anesthetized for appropriate examination, including lifting of the nictitating membrane, where the worms tend to hide. The limited clinical signs may be explained by the low number of eye worms in cats. In the previously mentioned study cats were harboring only 1–2 worms per eye (Malacrida et al., 2008). With a mean of 2.76 worms per eye, cats of this study seem to be more exposed to *Thelazia* infestations by trend. Furthermore, a single eye per cat was infested, with the exception of one cat having both eyes affected. In contrast, approximately one third of the dogs harbored eye worms in the left eye, one third in the right eye and one third in both eyes (Malacrida et al., 2008), suggesting that cat's eyes are at a lower risk of infestation than dog eyes.

If clinical signs are observed in cats, they seem to decrease with time of infestation, despite the persisting presence of the parasite; this reflects what was seen in the clinical follow-up study testing the efficacy of milbemycinoxime, where cats of the treated group did not clinically differ from the cats of the placebo group 7 days after treatment (Motta et al., 2012). It can therefore be assumed that prevalence in cats is generally underestimated, explaining

why previous reports of *T. callipaeda* in cats are anecdotal and why data about thelaziosis in cats and correlated risk factors are scarce.

For risk factor analysis, the study population has to be considered: a selection of cats which had to be anesthetized for different reasons was undertaken. The reasons for the veterinary examination were highly variable; based on the large number and on anamnestic data, the selected 2171 cats are assumed to represent a random cat population coming to a veterinary practice. In this study population, the number of male and female cats was equivalent. Unfortunately, the age was only known for 413 cats, not allowing therefore an evaluation of the whole population. Few pure-bred cats were examined, making assertions concerning breed predisposal difficult. Intriguingly, none of the pure-bred cats was *T. callipaeda* positive, while 11.1% of the cats with ocular illness were purebred, suggesting that possibly pure-bred cats such as Persians are more often examined for ocular problems due to anatomic conditions of their ocular ducts, but not for *Thelazia*-infestations. In dogs, a breed-size predisposal was defined, with lower prevalence rates in small sized breeds, and in young dogs (Malacrida et al., 2008). It was argued that prevalence in cats may be lower compared to dogs because of their small body mass index, making them less attractive for *Phortica* flies (the vectors for *T. callipaeda*), and because cats usually have intensive cleaning habits eliminating eye discharges. In fact, *Phortica* flies feed on fruits as other drosophilid species, but they additionally are attracted by eyes of humans and other mammals (Otranto et al., 2006; Roggero et al., 2010), showing a zoophilic behavior. Moreover, flies were described to be attracted by decaying fruits or slime fluxes and also by lachrymal secretions (Bächli et al., 2004; Otranto et al., 2006). Due to these particular feeding preferences, we may argue that *Phortica* flies are especially attracted by eyes with ocular discharge, which is often increased in animals with ocular problems. However, it remains debatable if the vector is particularly attracted by ocular discharge or if ocular discharge is a consequence of *Thelazia* infestation.

Significantly more male than female cats were found to be *Thelazia* positive, while in dogs contradictory results concerning sex predisposal were observed (Malacrida et al., 2008; Otranto et al., 2003). Since all cats of this study had outdoor access, it could be assumed that male cats are at higher risk for *T. callipaeda* infestation, possibly because of a wider roaming area and/or longer outdoor stay for hunting and territorial behavior (Finkler et al., 2011; Loyd et al., 2013), leading them to higher infestation risk.

Young cats may be at lower risk because of restricted contact with *Phortica* flies in their immediate surroundings, and/or because of the approximate 2 month duration after vector contact required for development of adult worms. The increasing number of cases with increasing age (in particular up to 6 years), may be linked to increased opportunities to vector contacts and to a potentially cumulative presence of the parasite in cats' eyes over time. Additionally, deworming frequency may decrease with increasing age of cats: veterinary actions such as vaccinations and desexing, which are often accompanied by administration of anthelmintic treatments, are frequent in

the first year of life, and anthelmintic treatments considered less necessary later on by the animal owners. Thus, established deworming protocols (www.esccap.ch) are not necessarily followed and according to this, a high number of adult cats may remain untreated. Furthermore, not all anthelmintics are efficacious against *T. callipaeda*: while intestinal helminths are eliminated applying compounds of the group of the benzimidazoles, quinolines, benzoles, depsipeptides, pyrimidines or macrocyclic lactones, only representatives of the latter ones were positively evaluated for efficacy against *T. callipaeda* (Ferroglio et al., 2008; Motta et al., 2012).

In respect of the relatively short time from infestation until adult parasites can be found, and that infestation can remain unnoticed for some time, it is not surprising that positive cats were diagnosed almost throughout the year. However, significantly more cases were diagnosed from June to December than during winter and spring, which is in contrast with the cases of ocular illness, which were more evenly distributed over the year. This difference indicates that the extent of the vector transmission season influences the seasonal detection of *T. callipaeda* positive cats. In southern Switzerland *Phortica* specimens were captured between April and October with the highest abundance of *Phortica* flies detected within an orchard in central Ticino (Roggero et al., 2010). In the studied Mendrisiotto region almost 20% is agricultural, which includes a considerable part of vineyard. This sustains the hypothesis that cases of *T. callipaeda* emerge in particular in areas attractive for the development of *Phortica* flies, as it has been shown in regions with fruit abundance, such as strawberry fields (Ruytoor et al., 2010).

Considering the potential development of uveitis or keratitis and correlated painful conditions due to the high innervation with sensory nerve fibers of the superficial cornea (Chan-Ling, 1989; Thomson et al., 2013) where *T. callipaeda* reside, and considering the potential role of cats in the transmission to the vector, it is important to detect such infestations for appropriate elimination. Currently, the therapy of *T. callipaeda* affected animals consists of the mechanical removal of the parasites and topical (Bianciardi and Otranto, 2005) or systemic (Motta et al., 2012) administration of macrocyclic lactones. Monthly anthelmintic treatments, which are advised as a control strategy for dirofilarioses and other helminth infections (see www.esccap.org), have already been suggested for animals living in areas endemic for *T. callipaeda* (Motta et al., 2012). Disease awareness among animal owners and veterinarians has likely increased ever since the first cases of thelaziosis were detected. Nevertheless, more frequent controls in all cats with outdoor access, in particular in male cats and in cats older than one year, are indicated for the detection of this underestimated disease, also in absence of clinical signs.

Acknowledgements

This study was supported by a grant (no 35080512) from Novartis Animal Health, Inc, and is part of the dissertation of Bruna Motta, veterinarian. Authors sincerely thank the veterinarians Ulli Scheu and Gionata Pedretti for the

precious collaboration finding cases, Günther Strehlau for statistical support and Lorraine Stapley for English review. All animal owners are highly acknowledged for their precious and indispensable contribution to the study.

References

- Anderson, R.C., 2000. *Nematode Parasites of Vertebrates. Their Development and Transmission*, 2nd ed. CAB International, Wallingford, pp. 404–407.
- Bächli, G., Vilela, C.R., Andersson Escher, S.A.S., 2004. *The Drosophilidae (Diptera) of Fennoscandia and Denmark*, Vol. 39. Brill, Leiden, New York, pp. 362.
- Bianciardi, P., Otranto, D., 2005. Treatment of dog thelaziosis caused by *Thelazia callipaeda* (Spirurida Thelaziidae) using a topical formulation of imidacloprid 10% and moxidectin 2.5%. *Vet. Parasitol.* 129, 89–93.
- Bussi eras, J., Chermette, R., Seillier, A.-M., 1996. Quelques parasitoses canines exceptionnelles en France. II – Un cas de conjonctivite parasitaire du chien, due   *Thelazia* sp. *Prat. M d. Chir. Anim. Comp.* 31, 83–85.
- Chan-Ling, T., 1989. Sensitivity and neural organization of the cat cornea. *Invest. Ophthalmol. Vis. Sci.* 30, 1075–1082.
- Clopper, C.J., Pearson, E.S., 1934. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 26, 404–413.
- Di Sacco, B., Ciocca, A., Sirtori, G., 1995. *Thelazia callipaeda* (Ralliet and Henry, 1910) nel sacco congiuntivale di un gatto di Milano. *Veterinaria* 9, 81–84.
- Dorchies, P., Chaudieu, G., Sim on, L.A., Cazalot, G., Cantacessi, C., Otranto, D., 2007. First reports of autochthonous eyeworm infection by *Thelazia callipaeda* (Spirurida Thelaziidae) in dogs and cat from France. *Vet. Parasitol.* 149, 294–297.
- Ferroglio, E., Rossi, L., Tomio, E., Schenker, R., Bianciardi, P., 2008. Therapeutic and prophylactic efficacy of milbemycin oxime (Interceptor) against *Thelazia callipaeda* in naturally exposed dogs. *Vet. Parasitol.* 154, 351–353.
- Finkler, H., Gunther, I., Terkel, J., 2011. Behavioral differences between urban feeding groups of neutered and sexually intact free-roaming cats following a trap-neuter-return procedure. *J. Am. Vet. Med. Assoc.* 238, 1141–1149.
- Koyama, Y., Ohira, A., Kono, T., Yoneyama, T., Shiwaku, K., 2000. Five cases of thelaziosis. *Br. J. Ophthalmol.* 84, 441.
- Loyd, K.A., Hernandez, S.M., Abernathy, K.J., Shock, B.C., Marshall, G.J., 2013. Risk behaviours exhibited by free-roaming cats in a suburban US town. *Vet. Rec.* 173, 295.
- Magnis, J., Naucke, T.J., Mathis, A., Deplazes, P., Schnyder, M., 2010. Local transmission of the eye worm *Thelazia callipaeda* in southern Germany. *Parasitol. Res.* 106, 715–717.
- Malacrida, F., Hegglin, D., Bacciarini, L., Otranto, D., Nageli, F., Nageli, C., Bernasconi, C., Scheu, U., Balli, A., Marengo, M., Togni, L., Deplazes, P., Schnyder, M., 2008. Emergence of canine ocular thelaziosis caused by *Thelazia callipaeda* in southern Switzerland. *Vet. Parasitol.* 157, 321–327.
- Miro, G., Montoya, A., Hernandez, L., Dado, D., Vazquez, M.V., Benito, M., Villagrasa, M., Brianti, E., Otranto, D., 2011. *Thelazia callipaeda*: infection in dogs: a new parasite for Spain. *Parasit. Vectors* 4, 148.
- Motta, B., Schnyder, M., Basano, F.S., Nageli, F., Nageli, C., Schiessl, B., Mallia, E., Lia, R.P., Dantas-Torres, F., Otranto, D., 2012. Therapeutic efficacy of milbemycin oxime/praziquantel oral formulation (Milbemax[®]) against *Thelazia callipaeda* in naturally infested dogs and cats. *Parasit. Vectors* 5, 85.
- Otranto, D., Cantacessi, C., Testini, G., Lia, R.P., 2006. *Phortica variegata* as an intermediate host of *Thelazia callipaeda* under natural conditions: evidence for pathogen transmission by a male arthropod vector. *Int. J. Parasitol.* 36, 1167–1173.
- Otranto, D., Dantas-Torres, F., Mallia, E., Digeronimo, P.M., Brianti, E., Testini, G., Traversa, D., Lia, R.P., 2009. *Thelazia callipaeda* (Spirurida Thelaziidae) in wild animals: report of new host species and ecological implications. *Vet. Parasitol.* 166, 262–267.
- Otranto, D., Ferroglio, E., Lia, R.P., Traversa, D., Rossi, L., 2003. Current status and epidemiological observation of *Thelazia callipaeda* (Spirurida, Thelaziidae) in dogs, cats and foxes in Italy: a “coincidence” or a parasitic disease of the Old Continent? *Vet. Parasitol.* 116, 315–325.
- Otranto, D., Lia, R.P., Buono, V., Traversa, D., Giangaspero, A., 2004. Biology of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts. *Parasitology* 129, 627–633.
- Otranto, D., Traversa, D., 2005. *Thelazia* eyeworm: an original endo- and ecto-parasitic nematode. *Trends Parasitol.* 21, 1–4.

- Poretti Suckow, D., Bottinelli, L., Paganetti, S., Stanga, M., 2012. *Annuario statistico ticinese 2012, Repubblica e Cantone Ticino, D.d.f.e.d.e., Ufficio di statistica*, ed. (Giubiasco, Società d'arti grafiche Veladini Lugano), 62.
- Rodrigues, F.T., Cardoso, L., Coutinho, T., Otranto, D., Diz-Lopes, D., 2012. Ocular thelaziosis due to *Thelazia callipaeda* in a cat from northeastern Portugal. *J. Feline Med. Surg.* 14, 952–954.
- Roggero, C., Schaffner, F., Bachli, G., Mathis, A., Schnyder, M., 2010. Survey of *Phortica* drosophilid flies within and outside of a recently identified transmission area of the eye worm *Thelazia callipaeda* in Switzerland. *Vet. Parasitol.* 171, 58–67.
- Rossi, L., Bertaglia, P.P., 1989. Presence of *Thelazia callipaeda* Railliet & Henry 1910, in Piedmont, Italy. *Parassitologia* 31, 167–172.
- Ruytoor, P., Dean, E., Pennant, O., Dorchie, P., Chermette, R., Otranto, D., Guillot, J., 2010. Ocular thelaziosis in dogs, France. *Emerg. Infect. Dis.* 16, 1943–1945.
- Shen, J.L., Gasser, R.B., Chu, D., Wang, Z.X., Yuan, X., Cantacessi, C., Otranto, D., 2006. Human thelaziosis – a neglected parasitic disease of the eye. *J. Parasitol.* 92, 872–875.
- Singh, T.S., Singh, K.N., 1993. Thelaziasis: report of two cases. *Br. J. Ophthalmol.* 77, 528–529.
- Soares, C., Sousa, S.R., Anastacio, S., Matias, M.G., Marques, I., Mascarenhas, S., Vieira, M.J., de Carvalho, L.M., Otranto, D., 2013. Feline thelaziosis caused by *Thelazia callipaeda* in Portugal. *Vet. Parasitol.* 196, 528–531.
- Thomson, S.M., Oliver, J.A., Gould, D.J., Mendl, M., Leece, E.A., 2013. Preliminary investigations into the analgesic effects of topical ocular 1% morphine solution in dogs and cats. *Vet. Anaesth. Analg.* 40, 632–640.
- Vieira, L., Rodrigues, F.T., Costa, A., Diz-Lopes, D., Machado, J., Coutinho, T., Tuna, J., Latrofa, M.S., Cardoso, L., Otranto, D., 2012. First report of canine ocular thelaziosis by *Thelazia callipaeda* in Portugal. *Parasit. Vectors* 5, 124.