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Review Article

Toxocara canis, *Toxocara cati* and *Toxascaris leonina* in wild and domestic carnivores

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Summary

Ascarididae nematodes of genera *Toxocara* and *Toxascaris* are of significant epizootic relevance among predatory mammals from families Canidae and Felidae. Localization of these nematodes in the definitive hosts, their morphology, as well as the measurements of eggs and adult worms are similar. Recently, molecular techniques have provided alternative approaches for the identification of ascarid species. A common feature of the life cycles of these generally monoxenous nematodes is the significant participation of small rodents. In case of *Toxocara* spp., the rodent plays the role of paratenic host but optional intermediate host for *T. leonina*. Several studies indicate co-occurence of both *T. canis* and *T. leonina* in domestic and wild canids as well as *T. cati* and *T. leonina* in felids. Although the infections of humans with *T. canis* and *T. cati* are common worldwide, larvae of *T. leonina* has the potential to cause human disease as emerging zoonosis.

Keywords: *T. canis*; *T. cati*; *Toxascaris leonina*; carnivores

Ascarididae nematodes - *Toxocara canis, Toxocara cati* and *Toxascaris leonina* are of significant epizootic revelance among predatory mammals from families Canidae and Felidae. Definitive hosts for *T. canis* include: dog (*Canis familiaris*), jackal (*C. aureus*), dingo (*C. dingo*), wolf (*C. lupus*), coyote (*C. latrans*), red fox (*Vulpes vulpes*), arctic fox (*V. lagopus*), fennec (*Megalotis zerda*), rarely feline species. Definitive hosts of *T. cati* are felines and include: cat (*Felis catus*), wild cat (*F. silvestris*), serval (*F. serwal*), lynx (*Lynx lynx*), cheetah (*Actinomyx jubatus*), puma (*Puma concolor)*, lion (*Panthera leo*), American leopard (*P. onca*), tiger *(P. tigris*), ocelot (*Leopardus pardalis*) and others. The definitive hosts of *T. leonina* are both feline and canine species. Several studies indicate co-occurence of both *T. canis* and *T. leonina* in domestic and wild canids as well as *T. cati* and *T. leonina* in felids (Labarthe *et al*., 2004; Dalimi *et al.*, 2006; Dubna *et al.*, 2007; Reperant *et al.*, 2007; Itoh *et al.,* 2011). For example, *T. canis* and *T. leonina* co-occured in 14 % of the red foxes population of Geneva, Switzerland (Reperant *et al*., 2007).
Localization of these nematodes in the definitive hosts, their morphology, as well as the measurements of eggs and adult worms are similar. The main difference concerns the construction of the caudal region of males; the tail of male *T. canis* and *T. cati* is characterized by a digitiform appendage and caudal alae. In contrast, the tail of male of *T. leonina* is conical with no caudal alae (Muller & Wakelin, 2002). There are also differences in the morphology of the anterior region. The cervical alae of *T. leonina* adults are longer and considerably narrower than those of *T. cati,* and the head of *T. leonina* resembles a spear, while the head of *T. cati* resembles an arrowhead (Taylor *et al.,* 2007). Also within genus *Toxocara* in the case of *T. canis* the cervical alae are elliptical and broad for *T. cati*.
The specific identification of ascaridoid nematodes of carnivores is a prerequisite for studying their epidemiology, population biology and systematics. Recently, molecular techniques have provided alternative approaches for the identification of ascarid species. Each parasite species has unique ribosomal DNA (rDNA) sequences which can be used as markers to distinguish them from closely related and/or morphologically similar species (Chilton *et al.,* 1995). Various studies have demonstrated that the first (ITS-1) and/or second (ITS-2) internal transcribed spacers (ITS) of nuclear ribosomal DNA (rDNA) provide reliable genetic markers for the identification of *T. canis*, *T. cati* and *T. leonina* (Jacobs *et al.,* 1997; Zhu *et al.,* 2000; Li *et al.,* 2007). Jacobs *et al*., (1997) first demonstrated that adults of *T. canis*, *T. cati* and *T. leonina* could be distinguished by their different ITS-2 sequences. The sequence differences (~26 – 50 %) between the species were significantly greater than the variation (0 – 0.6 %) within each species. These authors applied two PCR-based techniques, i.e. a two-step process

PCR-linked restriction fragment length polymorphism (RFLP) and a more simple PCR using specific primers. The PCR-RFLP could be used to delineate the three ascarid species by amplification of the ITS-2 and use of the restriction endonuclease *Hinf*I or *Rsa*I. Digestion with *Hinf*I differentiated *T. canis* and *T. cati* from other ascarids. The ITS-2 of the two *Toxocara* spp. remains undigested with this enzyme. Endonuclease *Rsa*I was used to distinguish between *T. canis* and *T. cati*, displaying a diagnostic banding pattern for each. Zhu *et al*. (1998) applying the PCR-RFLP and PCR-single stranded conformational polymorphism (SSCP) techniques confirmed that a nematode previously identified morphologically as *T. cati* was in fact a distinct species. Identification of species based on morphological criteria and host preference can have limitations, e.g. molecular analyses of ascaridoids in cats from Malaysia were supported by a subsequent morphological study (Gibbons *et al.,* 2001). The findings based on molecular analyses (ITS-1, ITS-2) showed that *Toxocara* sp. cf. *canis* from Malaysian cats represented a distinct species of *Toxocara*, and was named *T. malaysiensis,* being genetically more similar to *T. cati* than to *T. canis* (Gibbons *et al*., 2001). For the ITS-1 data alone, *T. canis* and *T. cati* were more similar to each other than either was to *Toxocara* sp. cf. *canis* (*T. malaysiensis*). Discovering *T. malaysiensis* has raised doubt about the specific identity of ascaridoids considered to represent *T. canis* from cats in other geographical regions (Gasser *et al.,* 2006) and provides a stimulus for the genetic characterization of *Toxocara* species from carnivores from other geographical origins of the world (Li *et al.,* 2006). A recent study has investigated the genotypes of adult *T. canis* and *T. leonina* living in the intestines of two different definitive hosts - dogs and foxes (Fogt-Wyrwas *et al.*, 2009). The results have not indicated an inward species genetic differentiation. The authors suggest that the parasite species examined do not have genetic barriers preventing them from settlement in different definitive hosts and thus have increased chance of survival.

Routes of infection of definitive hosts with these nematode species may vary. In addition to *per os* infection, there is the possibility of transplacental and transmammary transmission in the case of *T. canis* and transmammary for *T. cati*. Definitive hosts also become infected by ingesting rodent tissues containing the larvae of all three nematode species. A common feature of the life cycles of these generally monoxenous nematodes is the significant participation of small rodents. However, the role of rodents is different. After infection with embryonated eggs, larvae of *Toxocara* spp. migrate, then locate in the liver, lungs, heart, kidneys, muscles, and mostly in the brain of the host. While migrating larvae do not grow and develop significantly, reaching dimensions as follows: from an average length of 386 μm on the 1st day PI to 392 μm on the 60th day PI for *T. cati* and 406 – 429 μm for *T. canis* respectively (Okoshi & Usui, 1968a). Thus in this case, the rodent plays the role of paratenic host.

After rodent infection with *T. leonina*, larvae continue to develop for two or three months reaching average 877 μm in length in the 60th day PI (Okoshi & Usui, 1968a) and locate in the liver, lungs, kidneys, muscles but never in the brain (Prokopic & Figallova, 1982). The rodent plays a role of optional intermediate host in this case. Thus, it is considered that the cycles of nematodes of *Toxocara* and *Toxascaris* are non-strictly monoxenous (Reperant *et al.*, 2007).

There is also a different pattern of larvae migration of both genera in the definitive host. When embryonated eggs of *T. leonina* are used to infect the definitive host, there is a tissue phase in the gut wall before the larvae enter the gut lumen and mature. However, if, larvae first develop in the tissue of mice and rabbits and then the tissue is fed to cats or dogs, the tissue phase is eliminated and the prepatent period is reduced by about 10 – 15 days (Anderson, 2000). In *T. canis* and *T. cati*, eggs with third-stage larvae infect the host and there is the usual lung-tracheal or somatic migration. It is likely that dogs and cats usually get infected after ingesting eggs but embryonated eggs can hatch in various vertebrates and wander as visceral larval migrans or become encapsulated (Anderson, 2000).

The eggs of the three species are very similar in shape (subspherical) and dimensions. The shell is thick and composed of several layers. The eggs of *Toxocara* spp. have the pitted eggshell typical for the eggs of this ascarid genus. The pits on the outer layer of eggs of *T. cati* are smaller than the pits observed on the eggs of *T. canis*. The eggs of *T. leonina* are more translucent with a smooth shell surface. The outer layer is without striations or albuminous coat (Gonzales *et al.,* 2007). It is possible to differentiate relatively easy between the eggs of the genera *Toxocara* and *Toxascaris*. However, identification within the genus *Toxocara* is more complicated. According to Uga *et al.* (2000) measurements of egg dimensions has not been helpful in the differentiation of *Toxocara* species, because approximately 90 % of eggs measured were of similar size. Using scanning electron microscopy (SEM) it was possible to differentiate eggs of *T. canis* from *T. cati* based on their respective characteristic surface structures. Recently, a polymerase chain reaction (PCR) technique has been used for the differentiation of *T. canis* and *T. cati* eggs (Fogt-Wyrwas *et al.,* 2007, Borecka & Gawor, 2008). In some research based on coprological examinations of fecal samples the nematodes eggs are often identified only to the genus of *Toxocara* spp. instead of classification to the exact species (Szabova *et al.,* 2007; Sadzikowski *et al.*, 2009).

Egg resistance to both chemical and climatic factors influences egg viability in the environment over long periods of time. Soil type, ambient temperature and humidity are the main factors that determine the time it takes an egg to develop to the larvae L2/L3 stage (Sommerfelt *et al.,* 2006). Differences in the embryonating conditions and duration of larval development in eggs of *Toxocara* spp. and *Toxascaris* spp. have been observed. According to Feney-Rodriguez *et al.* (1988) *T. canis* eggs, don't embryonate in darkness although of *T. leonina* do so. Fully developed larvae appear in eggs of *T. canis* and *T. cati* within 2 – 3 weeks depending on environmental factors while larvae of *T. leonina* reach the infective stage in eggs

in 8 – 9 days at 27°C and in 3 days at 30°C (Kudryavtsev, 1974; cited in Anderson, 2000). Okoshi and Usui (1968b) examined the effect of various temperatures on the development of eggs of *T. leonina*, *T. canis* and *T. cati* and observed that the eggs of *T. leonina* could adapt to a greater variety of climate conditions than those of the *Toxocara* spp. When eggs were exposed to -15°C, the eggs of the two *Toxocara* species were dead after five days, while those of *T. leonina* were still alive after 40 days and when returned to 25°C almost all completed the development to the infective stage.

Co-occurence of *Toxocara* spp. and *T. leonina* in the definitive hosts is highly variable and depends on several factors: climate, environmental conditions, age of the hosts, and the season. This applies to infected wildlife as well as domestic animals. When investigating dogs and cats one must distinguish between animals obtained from their owners and living in shelters when compared with stray animals. In addition the dominance of one or the other species has been reported. When the prevalence of *T. canis* is very high e.g., 61.6 % in foxes in Great Britain (Smith *et al.*, 2003) or 81 % in Denmark (Willingham *et al.*, 1996) second species occurrence is very rare or not at all - 0.3 % and 0 % respectively. A similar pattern was noted in cases of high prevalence of *T. leonina* - 52.2 % and 4.4 % of *T. canis* in foxes from Spain (Criado-Fornelio *et al.,* 2000) as well as 47.1 % and 8.1 % in foxes from an area of the Slovak Republic (Antolova *et al.*, 2004). Also prevalence rates of *T. leonina* reported from dogs of seve-ral countries of the Balkan peninsula were lower than those of *T. canis* (Olteanu, 2000; Papazahariadou *et al.*, 2007) with the latest report on the rates of infection of 0.9 % and 75.7 %, respectively (Xhaxhiu *et al.*, 2011). Research of Meijer *et al.* (2011) on the endoparasites of arctic foxes (*Vulpes lagopus*) during two summers (2008 and 2010) showed the same pattern of nematode infection with *T. leonina* the most frequent parasite species found in 93 % and 65 % of the dens. In contrast, *T. canis* prevalence was considerably lower - 7 % and 30 %. Differences were also observed in felids infected with *T. cati* and *T. leonina*. For example, there are reports on the prevalence of these helminths - 37.5 % and 62.5 % in the Iberian wild lynx (*Lynx pardinus*) in Spain (Torres *et al.*, 1998) and 35.7 % and 8.8 % in cats from Brazil respectively (Labarthe *et al.,* 2004) (Table 1).

Vervaeke *et al.* (2005) suggest that the prevalence of *T. canis* and *T. leonina* in foxes is dependent on geographical location. Although the prevalences of both species were not determined the authors suggest that the prevalence of *T. canis* was higher. Both *T. canis* and *T. leonina* are present in northern Belgium. The prevalence of *T. canis* in northwest and central Europe varies widely, with high prevalences (ranging from 27 % up to 81 %) in southern Belgium (Losson *et al.*, 1997), Germany (Pfeiffer *et al.,* 1997), Austria (Lassnig *et al.,* 1998), Switzerland (Hofer *et al.,* 2000), Ireland (Wolfe *et al.,* 2001), the United Kingdom (Richards *et al.,* 1995) and Denmark (Willingham *et al.,* 1996) and lower prevalences in southern Europe (i.e. Spain, 4 – 6 %) (Criado-Fornelio *et al.,* 2000; Rodriguez & Carbonell, 1998; Gortazar *et al.,* 1998) and eastern Europe (i.e. Poland, 16 % – 17 %) (Luty, 2001; Gundłach *et al.* 1999). In contrast, the prevalence of *T. leonina* in northwest and central Europe is low (0 – 11 %) (Smith *et al.,* 2003; Richards *et al.,* 1995; Ballek *et al.,* 1992), whereas this nematode species is highly prevalent (25 – 67 %) in certain regions of Spain and southern France (Deblock *et al.,* 1987; Petavy & Deblock, 1980; Gortazar *et al.,* 1998).

The host habitat is the factor that seems to have a major impact on the incidence of carnivora infection with the nematodes of the genus *Toxocara* and *Toxascaris*. Research of Reperant *et al.* (2007) carried out in Switzerland showed that as many as 59.6 % of foxes from the rural environment were infected with *T. leonina* in contrast to only 8 % in urban area. The prevalence of that species decreased dramatically with the increase in the level of habitat urbanization. However, no significant influence of the degree of habitat urbanization was detected regarding the prevalence of *T. canis*. Soil contamination by *Toxocara* eggs has been found higher in urban than rural areas due to a higher density of domestic carnivores (Mizgajska, 1997).

T. canis is more often recorded in young animals up to 6 months of age than in adults also in female than in male dogs or foxes, which is associated with a specific larvae behavior in the host tissues. Szabova *et al.* (2007) found that in the Slovak Republic 53.2 % of dog pups up to 6 months, 37.5 % of older pups aged 6 – 12 months, and 18.8 % of dogs over 1 year of age were infected with *T. canis*. The possibility of transmission of *T. canis* larvae through the placenta may cause the high prevalence of infection during spring and summer in young foxes. A lower prevalence was observed in winter (Saeed & Kapel, 2006). A similar age related association in the infection pattern of domestic dogs was observed by Luty (2001). Trends of age dependent prevalence also occur in *T. cati*. For example, in Romania Mircean *et al.,* (2010) found a prevalence of infection of 30.8 % in young cats and 13.1 % in adult animals. However, in the case of *T. leonina* the infection is mostly observed in animals over 6 months of age. Szabova *et al.,* (2007) recorded this species in 3.6 % of dog pups up to 6 months of age and in 6.3 % of 6 – 12 months old dogs. Borecka (2003) has found *T. leonina* in 21.1 % of adult dogs but not in pups. Although *T. leonina* infection was found among 12-week old hound puppies in the United Kingdom (Fisher *et al.*, 2002).

In unnatural conditions for hosts and their parasites, such as zoos, where periodic treatment is carried out strictly, the transmission of *T. leonina* and *Toxocara* spp. occurs, *inter alia* by rodents (Okulewicz, 2008). Infected rodents, captured by carnivorous animals may contribute to *T. canis, T. cati,* or *T. leonina* infections resulting in significant epizootic problems. These nematodes have been recorded in wild Canidae and Felidae maintained in many zoos - for example in Berlin, Brno, and Wroclaw (Perec-Matysiak *et al.,* 2007). The common occurence of *T. cati* (64.3 %) in various species of Felidae at a zoo in Malaysia has been reported (Lim *et al.,* 2008). *T. leonina* is particu-

Table. 1. Prevalences of helminths in domestic and wild carnivores

Host species	Prevalence of parasite (%)			Localities	References
	T. canis	*T. cati*	*T. leonina*		
	61.6	-	0.3	Great Britain	Smith *et al.,* 2003
	81.0	-	0.0	Denmark	Willingham *et al*., 1996
	59.4	-	0.6	Denmark	Saeed & Kapel, 2006
	4.4	-	52.2	Spain	Criado-Fornelio *et al.,* 2000
Red fox	32.0	-	3.0	Germany	Loos-Frank & Zeyhle, 1982
(*Vulpes vulpes*)	4.5	-	31.8	Iran	Dalimi *et al*., 2006
	8.1	-	47.1	Slovak Republic	Antolova *et al.,* 2004
	12.5	-	42.9		Miterpakova *et al.,* 2009
	39.8	-	0.9	Poland (West)	Balicka-Ramisz *et al.*, 2003
	19.1	-	0.0	Poland (Central)	Borecka *et al.,* 2009
	54.4	-	0.0	Italy	Cerbo *et al*., 2008
	30.4	-	5.9	Kyrgyzstan	Ziadinov *et al*., 2010
Dog	6.0	-	32.5	Iran	Dalimi *et al*., 2006
(*Canis familiaris*)	6.2	-	0.9	Czech Republic	Dubna *et al.,* 2007
	21.9	-	7.3	Slovak Republic	Szabova *et al*., 2007
	16.3	-	0.6	Argentina	Soriano *et al*., 2010
Jackal	10.0	-	30.0	Iran	Dalimi *et al*., 2006
(*Canis aureus*)					
Wolf	13.5	-	3.8	Poland (North-east)	Kloch *et al*., 2005
(*Canis lupus*)	5.6	-	1.1	Poland (South)	Popiołek *et al.,* 2007
	21.2	-	13.5	Belorussian Polesie	Shimalov & Shimalov, 2000
Coyote	19.0	-	1.0	Canada	Bridger *et al*., 2009
(*Canis latrans*)					
Raccon dog	20.5	-	10.3	Belorussian Polesie	Shimalov & Shimalov, 2002
(*Nyctereutes procyonides*)					
Arctic fox	2.0	-	50.0	Iceland	Skirnisson *et al.,* 1993
(*Alopex lagopus*)					
Cat	-	34.5	0.0	Spain	Millan & Casanova, 2009
(*Felis catus*)	-	25.2	11.9	Brazil	Labarthe *et al*., 2004
	-	20.3	0.0	Romania	Mircean *et al*., 2010
	-	44.0	0.0	Iran (North)	Sharif *et al.*, 2010
	-	42.6	12.9	Iran (South)	Zibaei *et al*., 2007

larly common in large feline animals, for example, in the Wroclaw Zoological Garden it was found in 57.1 % of felids (lions, Bengal tigers, jaguars, pumas, lynx), while *T. cati* was found in only 14.3 % of felids (Okulewicz *et al*., 2002). Own research (not published, 2010) showed a persistent *T. leonina* infection in an Angolan lion (*Panthera leo bleyenberghi*) with up to 65 eggs in the field of view of direct microscopic smear preparations. Treatment with Valbazen and Advocate led to the initial elimination of the parasite, but subsequent examinations demonstrated its presence after two months. The persistent infections of *T. leonina*, ascertained in both the autumn-winter and spring-summer periods, in lion and leopard and snow panther in both the Warsaw and Plock Zoos has been reported by Bartosik and Górski (2010). Elimination of *T. leonina* and *Toxocara* spp. from the zoo environment is very difficult.

Lions in the Penjab Zoo (India) treated by chemotherapy (0 % prevalence) exhibited reinfection after thirty days of treatment (Singh *et al.*, 2006).

Helminth species presented in this paper are potential causative zoonotic agents. The infection of humans with *T. canis* and *T. cati* is common worldwide, causing mainly ocular larva migrans, visceral larva migrans and/or covert toxocariosis. Larvae of *T. leonina* can invade the tissues of laboratory animals and this species has the potential to cause human disease (Despommier, 2003). There is only one report of Beaver & Bowman, (1984) which describes a larva from the eye of a child in East Africa. This larva possibly represented a case of infection with some *Toxascaris* species. Further research should be carried out to better understand that emerging zoonotic agent.

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