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A survey on the prevalence of *Toxocara cati*, *Toxocara canis* and *Toxascaris leonina* eggs in stray dogs and cats' faeces in Northwest of Iran: a potential risk for human health

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Abstract. *Toxocara cati* and *Toxocara canis* target mainly cats and dogs respectively. However, *Toxoascaris leonine* can infect both cats and dogs. *T. cati* and *T. canis* are zoonotic parasites which can lead to visceral larva migrans and ocular larva migrans in humans. 200 faecal specimens of animals (100 stray cats and 100 stray dogs) were examined in this study from which 155 (77.5%) were reported to be infected by these parasites. Fifty cats from Azarshahr, fifty cats from Marand, and one hundred dogs from Tabriz were examined in the present study of which 45 (90%), 43 (86%), and 67 (67%), respectively, were reported to be positive at least for one of the parasites. Cats studied in Marand were under 1 and 1-2 years' old which showed significantly higher rates of infection with endoparasites (100%) compared to the cats which were above 2 years (30%; p<0.001). Findings showed that there was no significant difference between males and females (p=1.00). The rates of infection with *T. leonina* among cats from Azarshar showed a significant decrease with age (P<0.001). However, the rates of infection with *T. cati* was not significant at the same age groups (P>0.001). The rates of infection with *T. canis* among dogs less than 1 year (80%) were significant (P<0.05) in comparison to the rates reported for doges with 1-2 years (52.27%) and dogs more than 2 years old (57.69%). There were no significant differences between the prevalence of infections based on the host gender and urban sites (P > 0.001). From the public health point of view one can say that the high rates of infection with *T. cati*, *T. canis* and *T. leonina* in stray cats and dogs are important and critical. So it is necessary to implement appropriate measures and control strategies in order to prevent and control the helminth infections in stray cats and dogs in the areas survey in Iran.

INTRODUCTION

Stray cats and dogs have an essential role in the gastrointestinal helminthic parasites epidemiology and they can harbour a wide range of parasites which have a very important role in their health and also in human 's health (Hille *et al.*, 2014; Ramos *et al.*, 2013; Silaghi *et al.*, 2014). Stray cats and dogs can act as definitive hosts for many intestinal parasites, some of which can cause several zoonotic diseases such as visceral larva migrans (Bowman *et al.*, 2010; Richter *et al.*, 2014) and ocular larva migrans (Akao *et al.*, 2000). *Toxocara canis* and *Toxocara cati* are the two diseases caused by the ascarids of dogs and cats (Despommier, 2003). The species can also infect humans (Despommier, 2003). Larvae of *T. leonine* invade the tissues of animals. Some stages of *Toxocara* spp. life cycle occur outside the host, so the environment and paratenic hosts have an essential role in the temporal and spatial distribution of this parasite (Choi *et al.*, 2012). Adult *Toxocara* spp. and *T. leonina* usually locate in the upper small intestine of dogs and cats which act as definitive hosts and the female worms can produce up to

200,000 eggs per day which become infective within 3–4 weeks after passing out in the faeces (Schantz and Glickman, 1981). The infectious larvae in the eggs hatch after being ingested by dogs and cats and they penetrate the gut wall and after growing and moulting they return to the intestinal lumen and develop into mature worms. The main difference between *T. leonina* and other Toxocara is that the larvae do not migrate through the lungs and the whole of the life cycle takes place in the gut (Sprent, 1959). The most common way of infection of humans, especially children, is the eggs are ingested through infected faecal material (Strube *et al.*, 2013). In some rare cases, contact with soil containing infectious eggs-handling soil with an open wound or accidentally swallowing contaminated soil and eating raw or half cooked meat of intermediate host such as rabbit or lamb also has been reported as a cause of infection in humans. The animals allowed to be in public places also can pass the eggs of *Toxocara* spp. into the environment by their faeces and cause a risk to the population (Wolfe and Wright, 2003). Some of the determinative factors in this infection are the burden of parasite in stray cats and dogs; the season of the year; geographical region; administration of antiparasitic treatment and the factors which relate to population parameters (age, sex, breed) (Mircean *et al.*, 2010). Several studies have been conducted regarding the prevalence of endoparasites in stray cats and dogs by Necropsy or stool test (Torkan *et al.*, 2017; Kohansal *et al.*, 2017; Yakhchali *et al.*, 2017; Lopez *et al.*, 2017; El-Seify *et al.*, 2017; El-Dakhly *et al.*, 2017; Shahraki, 2016; Villeneuve *et al.*, 2015; Emamapour *et al.*, 2015; Ngui *et al.*, 2014; Mirzaei and Fooladi, 2012; Becker *et al.*, 2012; Borji *et al.*, 2011; Arbabi and Hooshyar, 2009; Davoust *et al.*, 2008; Zibaei *et al.*, 2007; Changizi *et al.*, 2007; Dalimi *et al.*, 2006; Jamshidi *et al.*, 2002). Few studies have been conducted on seroprevalence of *Toxocara* infection in human in Iran and other countries (Beiromvand *et al.*, 2018; Cook *et al.*, 2016; Negri *et al.*, 2013; Nurian and Amiri, 2009; Fallah *et al.*, 2007; Akhlaghi *et al.*, 2006; Sadjjadi *et al.*, 2000). There is very limited information about the contamination with *Toxocara* spp. and *T. leonine* eggs through stray dogs and cats' faeces in the northwest of Iran.

MATERIALS

Study areas

The study was conducted in Azarshahr (37°46' N and 45°85' E), Marand (38°17' N and 45°14' E) and Tabriz (38°4' N and 46°17' E) between April 2017 to February 2018. Azarshahr, Marand and Tabriz are located 1468 m, 1334m and 1348 m above mean sea level, respectively. These areas have a cold climate in winters and a mild climate in summers and the annual rainfall is approximately 100-388 mm.

Sample collection

The design of this study was cross sectional and 50 cats were sampled in each place. From Azarshahr the 50 cats consisted of 15 females (30%), 35 males (70%). From Marand another 50 consisting of 7 females (14%) and 43 males (86%) were sampled. The stray dog samples consist of 100 dogs (52% males and 48% females) from Tabriz. Stray cats trapped using baited cage traps with beef or chicken meat and collected from different areas according to the permission of the Environment Agency and veterinary Bureau of Azarshahr and Marand and then they were sent to the Department of Animal Biology, Faculty of Natural Science of Tabriz University. Some characteristics of the all cats and dogs were recorded including age (based on the teeth states), sex, breed, and weight (Eldredge *et al.*, 2008) and then they were divided in to 3 group based on their ages (Table 1). The samples of cat's faeces were collected one week after they being trapped in cages and faeces samples of dogs also collected from Pardis Animal Shelter of Tabriz, Iran.

Detection of eggs

In order to detect the eggs, faecal sample examination was done by direct and flotation method, using a saturated sugar solution which was described by Soulsby

Table 1. Animals studied based on age and gender in different regions

Animals	Area	Age group (year)						Sex	
		1>		1-2		2<		M	F
		M	F	M	F	M	F		
Cats	Marand	17	5	17	1	9	1	43 (86%)	7 (14%)
	Azarshahr	9	10	14	3	12	2	35 (70%)	15 (30%)
Dogs	Tabriz	16	14	21	23	15	11	52 (52%)	48 (48%)

(1982), with some modifications. Firstly, 2 grams of faeces sample were mixed with 10 ml water and then sieved through a 100 µm mesh sieve. The result solution was poured in to 12ml or 15ml centrifuge tube and after centrifuging at 1200 rpm for 5 minutes, the supernatant was added up to 1cm above the top of the tube and then mixed well and again was centrifuged at 1500 rpm for 10 minutes. Then the tube was filled with sucrose solution so that a small convex bubble formed and a cover glass was placed on the tube. Finally, the tube was centrifuged at 500 rpm for 5 minutes and the cover glass was put on a microscopic slide and was examined for the presence of *Toxocara* spp. and *T. leonina* eggs at a magnification of 40 and 100. The identification was carried out based on the method in Soulsby (1982).

Statistical analysis

The confidence interval of 95%, for statistical analysing, was calculated for each parasitic species. The correlation between host factors (age, sex and parasitism) and studied areas also was evaluated using Chi square test with SPSS software version 16.2.

RESULTS

Marand

43 (86%) cats from fifty cats examined were positive for at least one egg. The prevalence of *T. cati* and *T. leonina* were calculated as 86% and 12% respectively. Tables 2 and 3 show the number of infected cats and the prevalence of infection based on the cat's age and sex. The infections with endoparasites among cats under 1 year and 1-2 year were significantly higher compared to the cats which were above 2 years (30%; $p<0.001$). There was not any significant difference between male and female cats ($p=1.00$), however the rates of infections among females (100%) were higher than that of males (83.72%).

Azarshahr

45 (90%) cats were infected among the 50 cats investigated. The rates of infection prevalence were 90% and 30% for *T. cati* and *T. leonina*, respectively. The rates of infection with *T. leonina* were significantly high and showed a decrease with the age ($P<0.001$) but the rates of infection with *T. cati* were not significant at the same age groups ($P>0.001$). Although there was seen a difference in the rates of infection between male (88.57%) and female (93.33%), but there was not any significant difference between males and females (Table 2).

Tabriz

The overall rates of infection with *T. canis* and *T. leonina* among the stray dogs collected from Pardis Animal Shelter of Tabriz were 62% and 16%, respectively. The rates of infection with *T. canis* among dogs under 1 year (80%) were significantly higher compared to dogs with 1-2 years (52.27%) and dogs more than 2 years (57.69%), but the difference was not significant regarding the *T. leonina* among the same age groups ($P>0.05$). The infection rates in females (70.83%) were higher in comparison to the males (63.46%) is correct.

Table 2. The prevalence of helminthic eggs in the studied cats and dogs based on sex

Areas	Parasites eggs	Sex and prevalence (No. %)			Total (No. %)	
		Male	Female	P value	Infection	Mix. Inf.[a]
Marand		n=43	n=7		43 (86)	6 (12)
	T. cati	37 (86.04)	6 (85.71)	1.000		
	T. leonina	5 (5.81)	1 (14.28)	1.000		
Azarshahr		n=35	n=15		45 (90)	15 (30)
	T. leonine	9 (25.71)	6 (40)	0.333		
	T. cati	30 (85.71)	15 (100)	1.000		
Tabriz		n= 52	n=48		67 (67)	12 (12)
	T. canis	35 (67.30)	27 (52.25)	0.305		
	T. leonine	10 (19.23)	7 (14.58)	0.601		

a: Mixed infection.

Table 3. The distribution of helminthic eggs in the studied cats and dogs based on age

Areas	Parasites eggs	Age groups : No infected (%)			(EPG)			
		1>	1-2	2<	Significance X^2; P	T.I[a] No (%)	Mean	Max.[b]
Marand		n=22	n=18	n=10				
	T. cati	22 (100)	18 (100)	3 (30)	<0.001	43(86)	1.27	48
	T. leonina	0	6 (33.33)	0	<0.001	6(12)	7.14	15
Total of Inf. (No. %)		22(100)	18 (100)	3 (30)				
Azarshahr		n=17	n=19	n=14				
	T. cati	17 (100)	7 (89.47)	11 (78.57)	>0.001	45(90)	20.15	47
	T. leonina	13 (76.47)	2 (10.52)	0	<0.001	15(30)	6.14	10
Total of Inf. (No. %)		17 (100)	17 (89.47)	11 (78.57)				
Tabriz		n=30	n= 44	n=26				
	T. canis	24 (80)	23 (52.27)	15 (57.69)	<0.05	62(62)	7.16	35
	T. leonina	6 (20)	8 (18.18)	3 (11.53)	>0.05	16(16)	7.35	12
Total of Inf. (No. %)		28 (93.33)	24 (54.54)	15 (57.69)				

a: Total infection, b: Maximum.

DISCUSSION

Ascarididae nematodes of genera *Toxocara* and *Toxascaris* have significant epizootic importance among the predatory mammals of Canidae and Felidae, especially *Toxocara canis* and *Toxocara cati* are the most common zoonotic parasites in dogs and cats. The infection of humans can occur through infective eggs in contaminated soil, vegetables and fruits and then larva visceral migrants and ocular larva migrants can form. There are no significant differences in overall prevalence in studied areas, however some variations can be seen regarding the epidemiological aspects. The overall

prevalence of the infection with *T. cati* in stray cats of Azarshahr and Marand in this study (90% and 86% respectively) were higher compared to the previous studies conducted by examination of stool or autopsy in other cities of Iran, including Shiraz (42.6%) (Zibaei *et al.*, 2007), Isfahan (13%) (Jamshidi *et al.*, 2002), Isfahan (17.7%) (Torkan *et al.*, 2017), Kashan (13.3%) (Arbabi and Hooshyar, 2009), Mashhad (28.84%) (Borji *et al.*, 2011), North of Iran (8%) (Changizi *et al.*, 2007) and other countries such as; Egypt (8.23%) (El- Seify *et al.*, 2017), Canada (16.5%) (Villeneuve *et al.*, 2015), Malaysia (48%) (Ngui *et al.*, 2014), Germany (27.1%) (Becker *et al.*, 2012), Australia (3.2%) (Palmer *et al.*, 2008). The results of the present study is similar to the results of previous studies done by autopsy such as Azarshar (78%) (Hajipour *et al.*, 2016), Ahar (86.3%) (Yakhchali *et al.*, 2017). Poor hygiene, lack of anthelmintic drug used in stray cats, high humidity, and moderate temperatures can be among the reasons explaining the increased prevalence of *T. cati* in our studies and all the aforementioned factors were clearly detected in areas studied.

The rates of infection with endoparasites among cats with 1 and 1-2 year (100%) were significantly higher compared to the cats older than 2 years (30%; $p<0.001$). The rates of infection with *T. leonina* among cats from Azarshahr showed increase with age and were significantly high ($P<0.001$) but there was no significant increase in the rates of infection with *T. cati* at the same groups ($P>0.001$) and these results were in a good agreement with the results of the other studies (Becker *et al.*, 2012; Borji *et al.*, 2011; Sharif *et al.*, 2007). It is likely that infection can occur at any age, either by ingestion of eggs or tissues containing larvae, although the highest prevalence of infection occurs in kittens and young cats (Charleston, 1977). Nichol *et al.* (1981) in London and Shaw *et al.* (1983) in Perth, Western Australia, reported higher prevalence of *T. cati* in kittens than in adult cats, which is similar to the results of the present study. Swerczek *et al.* (1971) suggested that the high prevalence of *Toxocara* in kittens as due to the transmammary route of infection. O'lorcain (1994) showed that intra-uterine infection seldom occurs and that infection of *T. cati* mostly results from the ingestion of infective eggs, earthworms, cockroaches and/or rodents containing larvae in their tissues. Although, their immune system is not fully developed but it is able to generate a sufficient immunity (Gates and Nolan, 2009). The prevalence rate of *T. canis* found in this study (62%) was relatively similar to the rates reported from Gabon (58.5%) (Davoust *et al.*, 2008), however it was much higher than the rates reported from Iran (1.8%) (Kohansal *et al.*, 2017), (23.3%) (Shahraki, 2016), (29%) (Emamapour *et al.*, 2015), (29%) (Beiromvand *et al.*, 2013) and from Malaysia (34.4%) (Ngui *et al.*, 2014), Mexico (7.1%) (Lopez *et al.*, 2017). Variations in the routes of infection of definitive hosts with this nematode species can explain the high prevalence of these infections. On the other hand, the possibility of transplacental and transmammary transmission for *T. canis* and transmammary for *T. cati* also have been proposed. In addition, definitive hosts may become infected by ingesting rodent tissues containing the larvae of all three nematode species. Host habitat also is a factor that can affect the rates of infection with the nematodes of the genus *Toxocara* and *Toxascaris* among carnivores and all three areas studied in this research were suitable for developing of parasite eggs and there were not any antiparasite drugs for dogs in these areas. The results of the present study showed that the prevalence of *T. leonina* in stray cats of Marand, Azarshahr and stray dogs of Tabriz was 12%, 30% and 16% respectively, which were higher compared to the rates recorded in previously studies such as, Borji *et al.* (2011)(7.69%), Emamapour *et al.* (2015)(7%), Zibaei *et al.* (2007)(12.9%), Palmer *et al.* (2008)(0.3%), El-Seify *et al.* (2017)(8.32%), Yakhchali *et al.* (2017) Lopez *et al.* (2017)(5.5%), Mirzaei and Fooladi (2012)(0.9%), however the results of the present study are in disagreement with the results of the following studies: Dalimi *et al.* (2006) (32.53%) Beiromvand *et al.* (2013) (29%) on cats, El-Dakhly *et al.* (2017) (33.8%), and Senlik *et al.* (2006) (21.8%) about the dogs. One can attribute

the commonly occurrence of this parasite to the other invisible factors and intermediate hosts such as rodents and invertebrates, life cycle of *T. leonina*, and diet of stray cats. No study had been conducted on the seroprevalence of *Toxocara* infection in human in the studied areas. However, the seroprevalence rates of *Toxocara* identified in Ahvaz (Beiromvand *et al.*, 2018), Zanjan (Nurian and Amiri, 2009), Hamadan (Fallah *et al.*, 2007) and other countries such as Southeast Brazil (Negri *et al.*, 2013), Jamaica (Cook *et al.*, 2016) were 2%, 2.7%, 5.3%, 8.7% and 21.2%, respectively. Sadjjadi *et al.* (2000) showed that the seroprevalence rate of *Toxocara* infection among school children of Shiraz, Southern Iran, was 25.6%. They concluded that the incidence of human toxocariasis should be high, considering the degree of infection in dogs and cats in different countries as well as the high prevalence of *T. cati* and *T. canis* in cats and dogs in shiraz. In order to find out if there is a link between the human seroprevalence and the infection of stray dogs and cats with *Toxocara* in a region, both topics should be studied Simultaneously. However, it seems that there is a high level of human seroprevalence in areas where there is a high infection rate in stray dogs and cats.

CONCLUSIONS

High rates of infections with *Toxocara* spp. and *T. leonina* among stray cats and dogs have a significant importance in relation to the public health. These infections can cause several zoonotic diseases such as visceral larva migrants and ocular larva migrants. So appropriate control strategies and measures should be implemented in order to prevent and control these infections in this areas of Iran. Home-gardens should be fenced to prevent faecal contamination by dogs and cats. Vegetables and salads gathered from possibly contaminated gardens should be thoroughly washed and the consumption of raw or undercooked meat (paratenic hosts) that could harbour *Toxocara* larvae should be avoided. Geophagia should be brought to the attention of an appropriate health provider for treatment. Personal hygiene also should be upgraded by encouraging handwashing, especially prior to eating and discouraging hand to mouth activity at all times. Municipal ordinances to prevent pet dogs from entering parks and playgrounds and requiring owners to remove their pet's faeces from public areas should be considered.

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