



Serosurvey of anti-*Toxocara* antibodies and associated risk factors in domestic dogs and cats owners in Karaj, Alborz Province of Iran

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Mohammad Zibaei, Parisa Kiani Sefiddasht, Farzaneh Firoozeh, Abolfazl Miahipour, Saeed Bahadory. Serosurvey of anti-*Toxocara* antibodies and associated risk factors in domestic dogs and cats owners in Karaj, Alborz Province of Iran. Ann Agric Environ Med. 2022; 29(1): 50–55. doi: 10.26444/aaem/146318

Abstract

Introduction and Objective. Toxocariasis is a zoonotic parasitic infection with important public health considerations. The aim of the study was to assess the prevalence of anti-*Toxocara* species antibodies and associated risk factors in domestic dogs and cats referred by their owners to veterinary clinics located in Karaj, Alborz Province, Iran.

Materials and methods. A cross-sectional study involving 540 owners of dogs and cats was conducted between July – December 2020. A questionnaire administered by direct interviews was used to collect socio-demographic information and data on associated risk factors. Blood samples were collected and tested by indirect enzyme-linked immunosorbent assay (ELISA).

Results. The overall sero-prevalence of toxocariasis among the 540 participants was 16.7% (90 of 540). When participants included in the sample were classified by age, those aged 10–29 years demonstrated higher *Toxocara* infection prevalence than other groups (45.6%, 41 of 90). Univariate analysis revealed that the pet owners who had contact with soil [adjusted odds ratio (AOR)=7.61, 95% CI: 6.06–9.24, $P=0.028$], practiced handwashing after contact with dogs and cats (AOR=2.42, 95% CI: 1.15–4.85, $P=0.046$), and feeding the pets with raw meat (AOR=11.01, 95% CI: 5.21–19.43, $P=0.023$) had an increased risk of acquiring toxocariasis. The study showed that demographic characteristics such as age, gender, place of residence, education, and pet's habitats were not significantly associated with toxocariasis.

Conclusions. Given the findings and the progressive impact of toxocariasis in public health and its high prevalence in developing countries, including Iran, measures should be taken to inform the public about zoonoses and eliminate their putative transmission.

Key words

Toxocariasis, *Toxocara* infection, seroprevalence, owners of dogs and cats, diagnostic, veterinary clinics, Iran

INTRODUCTION

Toxocariasis is a zoonotic parasitic disease which can infect a large number of mammals including humans, and is a major burden for public health worldwide [1]. Dogs and cats can become infected if they contact with dirt and ingest dirt containing *Toxocara* species eggs. Although rare, infection can also occur through eating undercooked or raw meat from infected paratenic hosts such as mice, birds, and beetles [2]. Dogs and cats are the definitive host of *Toxocara canis* and *Toxocara cati*, respectively. One adult female of *Toxocara* can lay up to 200,000 unembryonated eggs per day, which are then shed in faeces into the environment. Taking in consideration the cases of high infestation in some dogs and cats, this number can rise up to millions of eggs per day. Egg embryonation begins in the environment, under proper temperature and humidity, and it takes between 2–5 weeks for the larvae to reach the infective stage. The embryonated eggs may infect different paratenic hosts which can harbour infective larva [3]. Humans can be infected

inadvertently by ingest contaminated soil with the feces of dogs or cats containing embryonated *Toxocara* eggs. The second-stage larvae are released into the small intestine, penetrate the intestinal wall, and are transmitted through the bloodstream to tissues and organs, but do not develop into adult worms. Hence, they involve a range of syndromes enclosing visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis (NT), and asymptomatic or covert toxocariasis (CT) [4].

There have been many epidemiological studies on the seroprevalence of *Toxocara* infection in the world. Since the serodiagnosis of human toxocariasis in the suspected people is established by detecting serum residual antibodies, the detection of classes of immunoglobulins, i.e. it has been proved that IgG is able to discriminate between active and passive (prior exposure to antigens) infections [5, 6].

A number of studies worldwide have described the extent of the high prevalence of *Toxocara* infection. Globally, toxocariasis is found in many countries, and prevalence rates can reach as high as 40% or more [7]. The reports indicates that between 118–150 million cats and ≥ 100 million dogs serve as definitive host of *Toxocara* around the world, shedding eggs and thus contributing to the public health risk of human infection [8, 9].

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Received: 27.07.2021; accepted: 31.02.2022; first published: 23.02.2022

The serological studies on the prevalence of *Toxocara* in the owners of dogs and cats in Karaj, Alborz Province in Iran are very limited. In a relatively similar study by Berenji et al. [10], a seropositivity rate of 20.43% and 1.07% were reported for anti-*Toxocara* antibodies by indirect ELISA technique in the owners of domestic dogs and cats and a control group, respectively. The researchers showed that a significant difference was observed between the prevalence of *Toxocara* seropositivity in study and control groups, but no significant difference found between the prevalence of toxocarasis in male and female groups. [10, 11].

OBJECTIVE

The aim of the study was to estimate and compare the prevalence of anti-*Toxocara* serum antibodies in the owners of dogs and cats referred to central veterinary clinics in Karaj, Iran. Considering to awareness of the serological status of the owners of domestic dogs and cats as one of the high-risk groups for *Toxocara* infection, they can provide useful information for this group and for community health-care,

MATERIALS AND METHOD

Ethical statement. The study was approved by the Ethics Committee at the University of Medical Sciences in Alborz, and informed consent obtained from the participants prior to data collection (IR.ABZUMS.REC.1399.218).

Study design, participants and location. The study involved the owners of dogs and cats referred to veterinary clinics (21 centres in 6 municipal districts) in Karaj, the capital of Alborz Province in Iran, between 1 July – 30 December 2020 (Fig. 1). The city is located at latitude 35° 49' north and longitude 50° 59' east, in the central part of the

country on the low-flat plain of the Karaj River. It is a sub-tropical region with long hours of sunshine, high temperature and humidity. Karaj has a total area of 162.14 km² with a population of approximately two million. The studied sites covered an area of 115.3 km² with 1,270,000 inhabitants [11].

Sample size. The sample size was determined using the single proportion population formula which indicates prevalence (P=40%), confidence interval (CI=95%), and the margin of error (d=5%):

$$n = (Z_{\alpha/2})^2 (P) (1-P) / d^2 = (1.96)^2 (0.4) (1-0.4) / (0.05)^2 = 0.921984 / 0.0025 = 369$$

After adding 45% (~171) for non-response or drop-out percentage, the appropriate sample size was 540. Considering the computer-based patient record, around 600 people were expected to visit the pet section clinics during the data collection period. Hence, the participants were selected by the systematic random sampling technique. All the owners of domestic dogs and cats, individuals (or caregivers) who volunteered and could give their consent participate were enrolled in the study. Respondents whose dogs or cats who had taken anti-parasitic drugs in the 2 weeks prior to data collection were excluded from the study.

Questionnaire data. A structured questionnaire was developed and used to collect socio-demographic information and clinical data on possible risk factor, which included age, gender, education, etc. The questionnaire was completed by trained interviewers who directly interviewed the participating the pet owners.

Blood collection and laboratory procedures. Five milliliters of blood were collected from the forearm vein by venipuncture and kept for 30 min at room temperature. The samples were conditioned in tubes with anticoagulant (EDTA), refrigerated, and sent to the Parasitology Laboratory of the School of

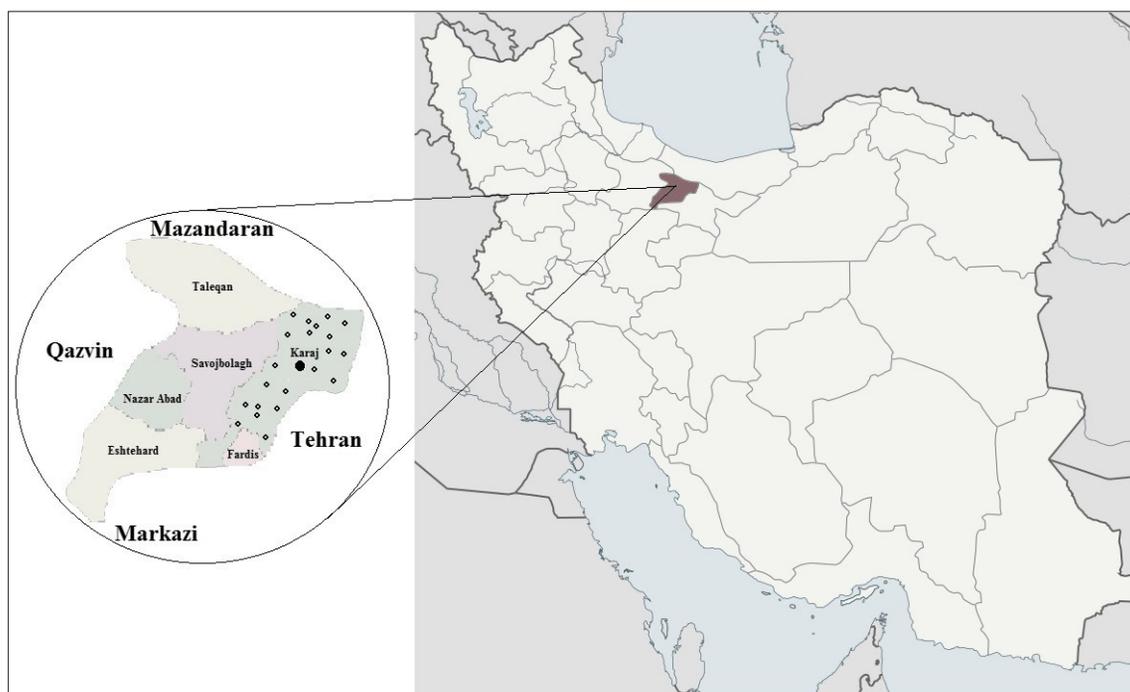


Figure 1. Map of Iran and Alborz province. The studied areas in Alborz province, where the owners of dogs and cats were tested for toxocarasis

Medicine, where they were immediately subjected to serological procedures. Serum samples were isolated were kept in -20°C prior to use.

Anti-*Toxocara* antibodies were detected by using the Enzyme-Linked Immunosorbent Assay (ELISA) kit (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany), which can achieve high diagnostic sensitivity and specificity (> 95%) and is considered the reference method for the serodiagnosis of toxocariasis [11]. Briefly, serum samples diluted 1:100 with IgG sample diluent were applied (100 µl/well) in duplicates and incubated at 3°C for 1 hour. After washing 3 times in washing solution, the plates were incubated with 100 µl/well of *Toxocara canis* protein A horseradish peroxidase-conjugate at 37°C for 30 min. For colour development, the plates were incubated with 100 µl of tetramethylbenzidine (TMB) substrate for 15 min at room temperature. The reaction was halted (100 µL/well) with stop solution for 15 min at room temperature. Optical density (OD) was determined at 450/620 nm for each well in a microplate reader (STAT-FAX-2100-OHAHIO-USA). The absorbance values were between 0.150–1.30 for cut-off control, <0.200 and < cut-off for negative controls and > cut-off for positive controls, as recommended by the manufacturer.

Data entry and statistical analysis. Statistical analysis to determine the association of positive owners with the studied variables was performed. Crude odds ratios (COR) and the adjusted odds ratio (AOR) with a 95% confidence interval (CI) were reported for the association of *Toxocara* antibodies between risk factors and infection of the pet owners. The level of significance was set at *P*-values less than 0.05.

RESULTS

Socio-demographic characteristics of study participants.

The study group comprised a total of 540 participants aged between 8–72 years. The median age was 28 years with 54.1% of participants (292 of 540) in the 10–29 years age group.

Seroprevalence of *Toxocara* infection. The overall seroprevalence of *Toxocara* infection among the participants was 16.7% (95% CI: 11.1–35.6%). When classified based on age groups, the prevalence of *Toxocara* infections in people aged 10–29 years was higher (45.6%) than other groups (*P*=0.051). The high prevalence of this disease among the mentioned age group indicates recent or current exposure to *Toxocara* eggs. Females had a higher seropositive rate (70 of 90; 77.8%) than males (20 of 90; 22.2%). Considering the findings of the Chi-square tests, no significant association was found between the prevalence of anti-*Toxocara* antibodies in the rural and urban residents (*P*=0.571) The observed higher risk of being seropositive among participants with high school educated (71 of 90; 78.9%), but 21.1% (19 of 90) of seropositive individuals had a university/college education level (*P*=0.712). The rates of seropositivity among the 6 districts of Alborz Province (14.4% in Azimieh, 21.1% in Gohardasht, 35.6% in Fardis, 7.8% in Shahinvila, 11.1% in Golshahr, and 10.0% in Mehrshahr) were not significantly different (*P*=0.970). Data analysis showed that there were no significant associations between toxocariasis and age group, gender, place of residence, level of education, and areas under study (Tab. 1).

Table 1. Sociodemographic characteristics of domestic dogs and cats owners referred to veterinary clinics in Karaj, Alborz Province, Iran, 2020

Characteristics	No. of samples (%)	No. of seropositive (%)	<i>P</i> -value
Age group (years)			
< 10	11 (2.0)	2 (2.2)	0.051
10-29	292 (54.1)	41 (45.6)	
30-49	167 (30.9)	22 (24.4)	
50-69	69 (12.8)	25 (27.8)	
≥ 70	1 (0.2)	0 (0.0)	
Gender			
Female	210 (38.9)	72 (80.0)	0.453
Male	330 (61.1)	18 (20.0)	
Residential area			
Urban	351 (65.0)	68 (75.6)	0.571
Rural	189 (35.0)	22 (24.4)	
Education			
High School	92 (17.0)	71 (78.9)	0.712
University/College	448 (83.0)	19 (21.1)	
Regions			
1 (Azimieh, North Karaj)	71 (13.1)	13 (14.4)	0.970
2 (Gohardasht, Karaj Northwest)	116 (21.5)	19 (21.1)	
3 (Fardis, South Karaj)	190 (35.2)	32 (35.6)	
4 (Shahinvila, Central Karaj)	46 (8.5)	7 (7.8)	
5 (Golshahr, West Karaj)	63 (11.7)	10 (11.1)	
6 (Mehrshahr, Karaj Southwest)	54 (10.0)	9 (10.0)	
Religion			
Muslim	540	100.0	

Factors associated with toxocariasis among participants.

The results of the present study showed that *Toxocara* infection was prevalent in the Alborz Province; therefore, assessing the association between toxocariasis and several potential predictor variables to determine risk factors for the infection was performed. In the multivariate logistic regression analysis, soil contact (AOR=7.61; 95% CI: 6.06–9.24; *P*<0.028), hand washing after contact with dogs and cats (AOR=2.42; 95% CI: 1.15–4.85; *P*<0.036), and feeding pets with raw meat (AOR=11.01; 95% CI: 5.21–19.34; *P*<0.023) had a statistically significant association with infection among the owners of domestic dogs and cats (Tab. 2). The analysis suggests that *Toxocara* infection is not significantly associated with other considered socio-demographic using the multivariate logistic regression analysis.

DISCUSSION

Toxocara species are zoonotic nematode commonly parasitizing dogs and cats worldwide with great importance to public health as the aetiological agent of human toxocariasis. The high rate of *Toxocara* seropositivity in the current study (16.7%, 95% CI: 11.1–35.6%) suggested that the toxocariasis is present among pet owners. Findings obtained in the current study were consistent with a previous study result from Mashhad, Iran (20.4%) [10]. The present seroprevalence was higher than findings in Slovakia (3.7%), The Netherlands

Table 2. Descriptive statistics of participants (N = 540)

Characteristics	Statistical analysis		Total	COR [†]	P- value*	AOR ^{**}	P- value
	Positive cases (%)	Negative cases (%)					
Age group (years)							
< 10	2 (2.2)	9 (2.0)	11 (2.0)	0.05 (0.00-0.43) ^{†††}	0.051		
10-29	41 (45.6)	251 (55.8)	292 (54.1)	0.02 (0.01-0.04)			
30-49	22 (24.4)	145 (32.2)	167 (30.9)	0.02 (0.01-0.04)			
50-69	25 (27.8)	44 (9.8)	69 (12.8)	0.32 (0.16-0.65)			
≥ 70	0 (0.0)	1 (0.2)	1 (0.2)				
Gender							
Female	70 (77.8)	140 (31.1)	210 (38.9)	0.25 (0.17-0.37)	0.453		
Male	20 (22.2)	310 (68.9)	330 (61.1)	0.00 (0.00-0.00)			
Soil contact							
Yes	72 (80.0)	243 (54.0)	315 (58.3)	7.59 (6.06-9.13) *	0.039*	7.61 (6.06-9.24) *	0.028*
No	18 (20.0)	207 (46.0)	225 (41.7)	0.00 (0.00-0.01)			
Residential area							
Urban	68 (75.6)	283 (62.9)	351 (65.0)	0.06 (0.04-0.08)	0.571		
Rural	22 (24.4)	167 (37.1)	189 (35.0)	0.02 (0.01-0.03)			
Education							
High School	71 (78.9)	21 (4.7)	92 (17.0)	0.91 (0.55-1.49)	0.712		
University/College	19 (21.1)	429 (95.3)	448 (83.0)	0.00 (0.00-0.00)			
Washing hands after contact with pets^o							
Yes	26 (28.9)	375 (83.3)	401 (74.3)	0.00 (0.00-0.01)			
No	64 (71.1)	75 (16.7)	139 (25.7)	2.31 (1.01-4.62) *	0.046*	2.42 (1.15-4.85) *	0.036*
Pets feeding							
Raw meat	59 (65.6)	62 (13.8)	121 (22.4)	11.43 (5.74-22.76)*	0.022*	11.01 (5.21-19.34)*	0.023*
Commercial food	12 (13.3)	50 (11.1)	62 (11.5)	0.06 (0.02-0.14)			
Home-cooked food	19 (21.1)	338 (75.1)	357 (66.1)	0.00 (0.00-0.01)			
Pets defecation area							
Fixed location	36 (40.0)	141 (31.3)	177 (32.8)	0.06 (0.04-0.11)	0.685		
Anywhere/other location	54 (60.0)	309 (68.7)	363 (67.2)	0.05 (0.03-0.07)			
Pets habitat^b							
Indoor	13 (14.4)	95 (21.1)	108 (20.0)	0.02 (0.01-0.04)	0.314		
Outdoor	77 (85.6)	355 (78.9)	432 (80.0)	0.05 (0.03-0.07)			

*Significant association; [†]COR: crude odds ratio; ^{**}AOR: adjusted odds ratio; ^{†††}CI: 95% confidence interval
^oDogs and cats; ^bHabitat of pets according to the information provided by their owners

(10.7%), Thailand (6.0%), Russian Federation (1.1%) and Austria (12.9%) [12–16]. On the contrary, the current results was lower than study findings of Mexico (26.2%), Taiwan (46.0%), Philippine (49.0%), and Ghana (53.5%) [17–20]. Smaller sample sizes in the previous studies may make account for such differences; for example, studies in Mexico, the Philippines and Russia recruited only 126, 75, and 90 participants, respectively [15,17,19]. A sampling error is a statistical error that occurs when a sample does not represent the entire population, a situation which may be observed in small sample sizes. This discrepancy may also be due to different populations and age groups of study participants, which contributes to differences in the seroprevalence of toxocarasis. On the other hand, laboratory methods and the diverse commercial kits used also have a significant role in the difference of *Toxocara* infection seroprevalence across studies. In the current study, serological evaluation was performed using the ELISA technique and NovaTec

Immunodiagnostica GmbH kit (Dietzenbach, Germany), which increases the detection rate of anti-*Toxocara* antibodies in sera.

Generally, several factors such as geographical, climatic, cultural, and some social and economic factors play a role in the prevalence of *Toxocara* infection. The areas in this study were 6 areas of Alborz Province, all of which were considered urban, and most of these areas have veterinary clinics. There are a large number of domestic dogs and cats owned in these urban districts. It was hypothesized that, according to previous reports and also the number of domestic dogs and cats in the area, influenced the rate of seropositivity in the current study [7, 11].

Among the 6 districts, Fardis showed the highest *Toxocara* positive rate (35.6%); however, there was no statistically significant difference between the 6 districts. This may be explained by the climatic, geographical and cultural similarities in the regions.

In the current study, there was no significant difference in the number of *Toxocara* positive sera between the young and the older age groups, which indicates the seroprevalence of *Toxocara* infection among the subjects aged 10–29 years (45.6%). Similar results were observed in a study from Greece between pet owners and non-pet owners [21].

The findings of the current study confirmed that female pet owners (77.8%, 70 out of 90) were more likely to be infected with *Toxocara* species than males (22.2%; 20 out of 90). Similar results have been also observed in previous studies [16,21]. It has been hypothesized that the reasons for such a high prevalence could be attributed to different behavioural attitudes, interest in keeping pets, and the close contact of the studied females with their dogs and cats. Long-term keeping of dogs and cats has been demonstrated to increase the risk of toxocariasis and *Toxocara* seropositivity rate in pet owners. The results of studies have shown that direct contact and keeping dogs and cats does not cause infection, but the conditions of keeping including diet, consumption of dry, canned or raw foods, as well as defecation conditions are very important [10, 22]. However, gender was not significantly associated with *Toxocara* seroprevalence in either the univariate or multivariate analysis in this survey.

Important factors affecting the seroprevalence of toxocariasis in Iran are the sub-tropical climatic conditions and moist soil, which cause an increase in the survival rate of *Toxocara* infective eggs [23]. In this study, more than half of the study subjects reported risk behaviour related to *Toxocara* infection, including contact with soil (58.3%). This fact suggests an important consideration in health education, that personal hygiene should be part of the educational curriculum for the owners of domestic dogs and cats.

A main finding of the study was the significant difference between seroprevalence in the studied urban people (68 of 90, 75.6%) compared with the rural population with toxocariasis. Dogs and cats in urban areas are a risk factor for human infection due to the possibility of soil contamination with parasitic eggs. A previous study by the authors showed that 36.4% of *Toxocara* eggs were recovered from soil samples in public parks of Karaj, Iran [23].

The pet owners with a low level of education (high school) had a relatively higher rate of *Toxocara* seropositivity, whereas a study performed in Karaj showed that the majority of pet owners with low education levels had a high contact risk with this parasite [11].

The current study revealed that *Toxocara* infection was most prevalent among the owners of dogs and cats in the studied population, and multivariable analysis revealed hand washing after contact with pets was statistically significant associated risk factors for toxocariasis. Humans acquire *Toxocara* by ingesting embryonated eggs, which can be found in public places worldwide, including Iran [10]. Several observational studies have indicated the impact of hand washing on the prevention of intestinal parasitic infections [23, 24].

Regarding modifiable risk behaviours, it seems that feeding pets with raw or uncooked meat is an important risk factor associated with toxocariasis in both the univariate and multivariate analyses. In the present study, the seropositive people who fed their pets with raw meat were more than 50% at higher risk of acquiring *Toxocara* infection, compared with those who fed their pets commercial or home-cooked food. This is supported by Baneth et al. (2016) who revealed

that the risks of being infected by *Toxocara* are increased by carnivores that consumed uncooked or raw meat [24]. One of the infective stages of many *Toxocara* species, encapsulated infective larvae, can be acquired from raw or uncooked meat of auxiliary or paratenic hosts, which causes the parasites to be swallowed when eating [25].

The results of this study illustrate that the effects of control measures to prevent the spread of *Toxocara* infection are effective in the study area. However, the prevalence of toxocariasis was high in the participants whose pets did not defecate in a specific location, but had scattered faeces, although the difference was not statistically significant. The results of a previous study indicate the low awareness of pet owners about the zoonotic parasite threats to humans. Prevention of initial environment contamination with *Toxocara* species eggs, which includes defecating in public areas and private households, is vital [26].

CONCLUSION

Toxocariasis remains a seroprevalence infection among the owners of domestic dogs and cats in the urban areas of Karaj, Iran. The findings of this study showed that contact with soil, inadequate hand washing after contact with pets, and feeding pets with raw or uncooked meat, were statistically associated with *Toxocara* infection. Due to the considerable zoonotic parasitic disease prevalence and risk factor-related behaviours among pet owners in the studied area, a combination of measures for appropriate management should be taken to reduce the prevalence of *Toxocara* infection in this community, which should include personal and environmental health education, and the development of awareness strategies and prescription of medication for treatment.

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