



# Sero-epidemiology of *Toxocara canis* infection in people attending four educational and therapeutic centres in Alborz Province, Iran

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## Abstract

**Introduction and Objective.** Toxocariasis is a common but neglected zoonotic disease caused by the larval stage of *Toxocara* species. The aim of this study is to highlight the sero-epidemiology of *Toxocara canis* infection in the general population of Alborz Province, Iran.

**Materials and method.** Anti-*Toxocara canis* antibodies were tested in 234 studied inhabitants by enzyme-linked immunosorbent assay (ELISA). Demographic characteristics and consent were obtained from each individual.

**Results.** In total, 33 (14.1%) of the 234 participants (mean age 19.6±8.7 years) had anti-*Toxocara canis* antibodies. Prevalence of infection was higher in males (16.2%) than females (12.4%). High-titer antibodies were most prevalent among the subjects aged ≤ 20 years, and showed statistical significance (p=0.001). There was significant association between the presence of *Toxocara* antibodies and the place of residence, keeping dog at home, and contact with contaminated soils (p<0.05). This is the first report of the sero-prevalence and contributing factors for *Toxocara canis* infection in the general population in Alborz Province.

**Conclusions.** These data confirm the importance of diagnosing human toxocariasis in this region. The findings, in addition to the hygienic status of the population, can be representative of the environmental and animal contamination. Necessary education to avoid contact with suspicious dog and non-contact with soil from infancy should be considered as preventive measures for *Toxocara* infection.

## Key words

*Toxocara canis*, diagnosis, toxocariasis, sero-epidemiology, ELISA, Iran

## INTRODUCTION

Toxocariasis is an important helminthic zoonosis due to the human infection by larvae of *Toxocara canis* (*T. canis*), the common nematode of dogs, and less frequently by the cat nematode *Toxocara cati* (*T. cati*) [1]. The human infection is acquired through the ingestion of embryonated eggs found in soil, contaminated vegetables, on dirty hands or undercooked meat of the paratenic hosts that hatch in the host's intestine [2]. There is a varied clinical spectrum of toxocariasis in humans, ranging from asymptomatic infection to severe organ injury, with symptoms related to the migration of third-stage larvae of *Toxocara* species via the bloodstream to the internal organs. The expatriation of parasitic larvae into the viscera or other organs causes the symptomatic form of infection, in the state situation manifestations include three major complications of visceral and ocular larvae migration, and neurotoxocariasis [3]. The majority of the population involved in *Toxocara* infection are children, but the detection

of even previous infections in adults is valuable because it not only determines the prevalence of infection in individuals in the area, but is also a good indicator for environmental health assessment, animal infection status, and from the preventive planning perspective [4].

Enzyme Linked Immunosorbent Assay (ELISA), although a relatively old technique, is still a highly sensitive and reliable test in the diagnosis of some infections, one of which is *Toxocara*. ELISA based on *T. canis* excretory-secretory (TcES) antigen is routinely used. TcES-ELISA sensitivity and specificity is estimated approximately 78% and 92% respectively [5, 6]. To-date, most studies have included a specific population in evaluation of toxocariasis prevalence: in Iran, eosinophilia individuals under 14 years of age [7]. However, it should be noted that this is not always the case, and eosinophilia is not necessarily in all *Toxocara* infections, all age groups should also be included. Risk factors that may contribute to *Toxocara* infection should be considered in addition to age, gender, primarily exposure rate, and contact with contaminated soil and/or an infected animal, personal health situation; additionally, the economic and education status, as well as other demographic variables, should be included.

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This is the first sero-epidemiological study to be performed in this region, although *Toxocara* epidemiological research, including ocular toxocariasis, soil contamination by *Toxocara* spp., and paratenic hosts of *T. canis* have been carried out previously [8, 9].

## OBJECTIVE

Due to the importance of this helminth zoonosis and lack of data about its prevalence in the studied area, a sero-epidemiological study was conducted with the aim of assessing the prevalence of human *Toxocara* infection, and the epidemiological factors associated with the spread of the disease.

## MATERIALS AND METHOD

**Ethical consideration.** Informed consent was obtained from all participants. The study design, including its ethical aspects, was reviewed and approved by the Ethics Committee of Alborz University of Medical Sciences (IR.ABZUMS.REC.1398.107).

**Study Area.** Alborz Province centered in Karaj (35°49'57", 50°59'29"), is located in the north of Iran (Fig. 1). This region with a population of around 3 million is one of the five most populous cities in Iran. The Alborz Province climate, to some extent, is cooler than the neighbouring provinces, with a mean temperature in this area of 15.1°C, and an annual rainfall of 260 mm ([https://en.wikipedia.org/wiki/Alborz\\_Province](https://en.wikipedia.org/wiki/Alborz_Province)).

**Serum samples.** A total of 234 sera samples were collected from randomly examined individuals (129 females, 105 males) attending four educational and therapeutic centres in Alborz Province, Iran, between October 2020 – March 2021. Of the 234 people who gave blood samples, 141 lived in urban and 93

in rural areas. The demographic details of the respondents and data relating to their demographic characteristics, including age, gender, place of residence, occupation, history of disease, and level of education, were recorded. After consent was given by each participant, their demographic details and infection risk factors regarding gender, age, contact with dogs and cats or their feces, income and education, as well as urbanization level, also were obtained. None of the participants had specific clinical signs of the disease.

**TcES based Enzyme Linked Immunosorbent Assay.** In the Parasitology Laboratory of the School of Medicine, Alborz University of Medical Sciences, peripheral venous blood samples (~ 5 mL) were taken under sterile conditions from 234 randomly examined individuals, and the sera of these samples were separated by centrifuge at 2,500 rpm, aliquot, and stored at -20°C until analyses were carried out. Anti-*Toxocara* antibodies were detected by commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany), according to the manufacture's instructions.

**Statistical analysis.** Statistical tests were performed to evaluate the association between study variables and results. Demographic characteristics and possible risk factors for *Toxocara* infection were assessed by Chi-square tests with a 95% confidence interval (95% CI). All data analyses were carried out with SPSS 21.0 statistical software.

## RESULTS

Of the 234 serum samples, 33 (14.1%) people IgG against *T. canis* infection were tested positive by the TcES-ELISA kit. The distribution of the titers is shown in Table 1. Seroprevalence was higher in males – 16.2% (17/105), than females – 12.4% (16/129), respectively (Tab. 2). The prevalence

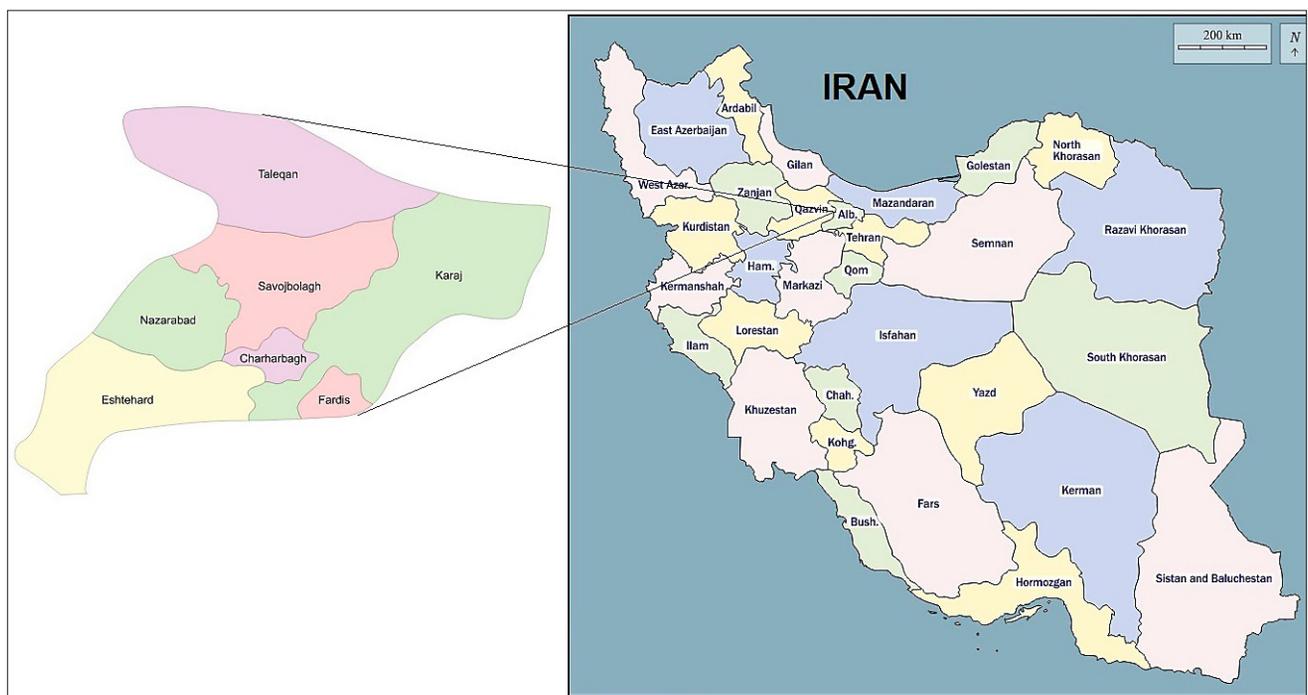


Figure 1. Location of studied sites

**Table 1.** Distribution of *Toxocara* serologic titers by ELISA

Serologic titers	People	
	Number	Percent
1:160	3	1.3
1:320	4	1.7
1:640	22	9.4
1:1280	3	1.3
1:2560	1	0.4
Negative	201	85.9
<b>Total</b>	<b>234</b>	<b>100</b>

**Table 2.** Characteristics of people investigated by enzyme-linked immunosorbent assay for human toxocariasis

Factors	Total	Statistical analysis		(P value) <sup>†</sup>
		Seropositive (%)	Seronegative (%)	
<b>Age group (years)</b>				
Less than 20	76 (32.4)	17 (22.4)	59 (77.6)	0.001
21–40	66 (28.2)	10 (15.2)	56 (84.8)	
41–60	75 (32.1)	4 (5.3)	71 (94.7)	
More than 60	15 (7.3)	2 (13.3)	13 (86.7)	
<b>Gender</b>				
Female	129 (55.1)	16 (12.4)	113 (87.6)	0.746
Male	105 (44.9)	17 (16.2)	88 (83.8)	
<b>Soil contact</b>				
Yes	82 (35.1)	21 (25.6)	61 (74.4)	0.049
No	152 (64.9)	12 (7.9)	140 (92.1)	
<b>Pet keeping</b>				
Yes	32 (13.7)	9 (28.1)	23 (71.9)	0.031
No	202 (86.3)	24 (11.9)	178 (88.1)	
<b>Education</b>				
High school diploma	222 (94.9)	18 (8.1)	204 (91.9)	0.404
More than high school	12 (5.1)	5 (41.7)	7 (58.3)	
<b>Income threshold<sup>‡</sup> (monthly)</b>				
≤ 2000	116 (49.6)	17 (14.7)	99 (85.3)	0.629
2001–3999	69 (29.5)	9 (13.1)	60 (86.9)	
≥ 4000	49 (20.9)	3 (6.1)	46 (93.9)	
<b>Residency</b>				
Urban	141 (60.3)	12 (85.7)	2 (14.3)	0.024
Rural	93 (39.7)	2 (2.1)	91 (97.9)	

<sup>†</sup>Significance by the  $\chi^2$  test; <sup>‡</sup>Dollars (US)

rate in the age group  $\geq 60$  years was the lowest among the other age groups 7.3% (15/234); overall, the age factor was statistically significantly associated with infection ( $p=0.001$ ), followed by contact with dogs ( $p=0.031$ ) and soil ( $p=0.049$ ) variables, respectively. On the other hand, among the studied variables and possible risk factors, was significantly associated with *T. canis* infection, but the rest of them, such as education and income level, as well as urbanization, were not significantly related to infection (Tab. 2).

Antibodies to *T. canis* were surveyed in 234 subjects aged 5–79 years (mean  $19.6 \pm 8.7$  years), with an overall seroprevalence rate of 14.1%, which was substantially higher among those aged

$\leq 20$  years ( $n=17$ , 22.4%) than in older subjects aged 21–40 years ( $n=10$ , 15.2%, 41–60,  $n = 4$ , 5.3%), and  $> 60$  years ( $n=2$ , 11.8%;  $p=0.001$ ). High-titer antibodies were most prevalent among those in the age group  $\leq 20$  years, suggesting that recent or current exposure to *Toxocara* is most common in this age group. Seropositivity was more prevalent in males (16.2%) than in females (12.4%), and showed no statistical significance ( $p=0.746$ ). Some demographic characteristics, such as education, were not significantly associated with the presence of *Toxocara* antibodies in unadjusted analysis (Tab. 2).

## DISCUSSION

Human toxocariasis is an important health problem in the developing countries. Immunodiagnostic tests against toxocariasis are valuable for compiling epidemiological data, and used as additional tools in clinical diagnosis to detect specific antibodies with great precision. The socio-economically disadvantaged and demographic factors may lead to increase in *Toxocara* sero-prevalence which is widely recognized as a parasitic disease worldwide, and may be much more common than previously thought. Since the clinical symptoms of toxocariasis are of limited value in the differential diagnosis, serological tests are of considerable importance in the detection of *Toxocara* infection [5]. Most epidemiological studies on human toxocariasis in Iran have been carried out on children and the sero-prevalence ranged between 1.4% – 29.5% [7].

To the best knowledge of the authors, the current study is one of the first to present serological data of *T. canis* infection among the population of the Karaj district in Iran. The frequency of *Toxocara* infection in people participating in the study was 14.1%. A high sero-prevalence (9.4%) was observed in 22 studied people with elevated antibodies titers of 1:640. The interpretation of sero-prevalence data remains very difficult due to the use of different cut-off titers by various researchers, and the difficulty in evaluating the relationship between titer level, infection, and clinical findings of the disease [10].

The prevalence of human *Toxocara* infection confirms that Karaj has a comparable infection rate with other areas in which toxocariasis is considered to be highly endemic. In a study by Zahedan in southeast Iran, 11.3% of the students with cough were seropositive [11]. A case control-study showed that the prevalence of toxocariasis and *Toxocara* antibodies was 14.7% among multiple sclerosis patients in Khorram Abad, southwest Iran [7]. The prevalence of *Toxocara* infection in the present study is similar to the rates reported in previous studies, although the rate of *Toxocara* infection in the present study was slightly lower than the rates in other countries, including Taiwan (57.5%) [12], Philippines (49.0%) [13] Korea (51.2%) [14], and Thailand (58.2%) [15], whereas the rate was higher than those in some other parts of the world, including Turkey (8.0%) [16], and in the Czech Republic (3.6%) [17].

Given that pet keeping was a putative risk factor, the findings of the current study showed that the highest seropositive individuals had been exposed to dog contaminated faeces (28.1%), while the lowest incidence occurred with the lack of contact to dog faeces (11.9%). However, human toxocariasis is an important and common zoonotic helminthes infection worldwide [2].

The current study shows that the seropositivity rate was not significantly higher in males (16.2%) than females (12.4%). The reasons for such a high prevalence could be attributed to different behavioural attitudes, and the close contact of males with their dogs. The results of the study provide an indication of the prevalence of toxocariasis among the subjects aged less than 20 years (22.4%). According to a previous study, the prevalence of toxocariasis is in the age group of young children (Arefkhah et al., 2020). Several risk factors for toxocariasis have been identified in the human population, but inconsistent results are abundant. Gender, for example, was suggested to be associated with both increased and decreased risk of infection. Young age and poor sanitation are additional factors contributing to *Toxocara* exposure in some communities [19].

In the current study, similar to previous studies on *Toxocara* sero-prevalence [20], it was found that *Toxocara* seropositivity is higher in people with a lower income. The reasons for such a high prevalence could be attributed to different behavioural attitudes, lack of sanitation, malnutrition, and a low-quality of life associated with poor economic conditions. However, these associations were not statistically significant in the multivariate analysis.

A main finding of the study was the significant difference to *Toxocara* between sero-prevalence in the rural population compared with the urban population, so that a significant difference was found between rural life and urban life (22.6% vs. 7.5%, respectively). The current study confirms that inhabitants of rural communities may be heavily exposed to *Toxocara*. The presence of an unrestrained dog population and adequate climate and environmental conditions for egg survival contribute to the high seroprevalence rate observed. Some researchers have found that the risk of developing *Toxocara* infection is higher in rural areas than in urban areas [19]. In earlier studies, some provinces in Iran, such as East Azerbaijan, Ahvaz, and Mashhad, demonstrated a significant difference with the place of living. It was observed that most of the affected people were in soil contact in rural areas [8].

An important result of the presented study is the high seropositivity rate (28.1%) of toxocariasis in people who keep dogs, with the probable reason being the presence of dogs in the household and their close contact with *Toxocara* eggs.

The seropositivity rate was not affected by level of education, although there were significantly more seropositive patients who did not have college education. The high prevalence of antibodies titer in people with primary education may be associated with socio-economic status, an observation that suggests that the population with primary education were more likely to be exposed to the parasite.

It is worth mentioning that the current study has some limitations which constrained the findings: 1) the clinical and laboratory parameters were not fully investigated in all patients; 2) no correlations were shown between eosinophilia and toxocariasis, and 3) it seems likely that history taking for risk factors may had been incompletely performed; therefore, in future, thorough evaluation with laboratory parameters and history taking should be undertaken in cases where toxocariasis is suspected.

## CONCLUSIONS

In spite of numerous studies conducted on this disease, evidence-based and standardized approaches are still needed to define appropriate strategies for the epidemiological evaluation, immunodiagnosis, and clinical management of *Toxocara* infection, among others.

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