

Seroprevalence and Risk Factors of *Toxocara canis* Infection in Children (2–15 Years Old) Referred to Health Centers of Lorestan Province, Iran

Hossein Mahmoudvand¹ Nadereh Taeae² Farzad Ebrahimzadeh³ Mojgan Sadat Mirhosseini⁴
Mojgan Faraji²

¹Department of Medical Parasitology, Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

²Department of Pediatric, Lorestan University of Medical Sciences, Khorramabad, Iran

³Department of Biostatistics, Faculty of Health and Nutrition, Lorestan University of Medical Sciences, Khorramabad, Iran

⁴Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran

Address for correspondence: Nadereh Taeae, MD (Assistant Professor), Iran, Islamic Republic of (e-mail: taeae_n47@yahoo.com).

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Abstract

This study aims to evaluate the seroprevalence and risk factors for *Toxocara canis* infection in children (2–15 years old) referred to health centers of Lorestan province, Iran. This cross-sectional study was performed from August 2016 to March 2017 on 316 children. All serum samples were tested using the commercially available anti IgG-*Toxocara* kit (IBL, Germany) according to manufacturer's instructions. Of the 316 children, 14 (4.4%) tested seropositive for anti-*T. canis* IgG antibody. The variables used to evaluate association between risk factors and status of anti-*T. canis* IgG antibodies were age, gender, area of residence, eosinophilia, contact with dogs, and consumption of raw or unwashed vegetables and fruits. Risk factors that were significantly related to *T. canis* seropositivity included living in rural regions ($p = 0.018$) and contact with dogs ($p = 0.001$). However, other demographic and risk factors did not demonstrate any association with *T. canis* seropositivity. To conclude, we found that *T. canis* infection is prevalent among children (2–15 years old) referred to health centers of Lorestan province, Iran with an overall seroprevalence rate of 4.4%. These findings may be a warning for health centers to pay special attention to toxocariasis among children (2–15 years old) and design screening programs for its prevention.

Keywords

- ▶ toxocariasis
- ▶ ELISA
- ▶ IgG
- ▶ Lorestan

Introduction

Human toxocariasis is a helminthic zoonotic disease caused by the larval stage of *Toxocara* spp. To date, *T. canis* and *T. cati* are identified as contributory agents of human toxocariasis that inhabit the small bowels of dogs and cats as definitive hosts, respectively.¹ Typically, humans may be infected by direct contact with soil contaminated with the feces of dogs with toxocariasis and eating embryonated eggs through

contaminated water and food, although ingestion of chicken and cow liver is considered an uncommon route of infection.² Although human toxocariasis in immunocompetent individuals is commonly asymptomatic, serious symptoms and complications can occur due to vital organ damage as a result of migrating larvae.^{1,3} Accordingly, clinical complications of human toxocariasis are identified as visceral larva migrans (VLM), ocular larva migrans (OLM), and neurologic and complex toxocariasis.¹ The identification of human

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toxocariasis is based on a serological test, enzyme-linked immunosorbent assay (ELISA), by using excretory–secretory antigens from *Toxocara* larvae. It is extremely hard to determine infective *Toxocara* larvae in various organs and biopsy samples.⁴

Children in the first and second decades of life are at far more risk of toxocariasis due to contact with the definitive host (especially dogs) and lack of hygiene standards in the consumption of food and water; therefore, it is crucial to study the prevalence of anti-*Toxocara* antibodies in children in this age range.⁵ Based on previous studies, the seroprevalence of infection with toxocariasis in different geographic regions varies from 2 to 90% around the world.^{2,6} According to the best of our knowledge, there is no study on seroprevalence of human toxocariasis among the children of Lorestan province, western Iran. Therefore, the present study aims to evaluate the seroprevalence and risk factors for *T. canis* infection in children (2–15 years old) referred to health centers of Lorestan province, Iran.

Materials and Methods

Ethics

This study was approved by Ethics Committee of the Lorestan University of Medical Sciences, Khorramabad, Iran on March 15, 2017. In addition, a written informed consent was obtained from the parents of the children before blood sampling.

Study Design and Sample Collection

This cross-sectional study was performed from August 2016 to March 2017 on 316 children (aged 2–15 years) referred to health centers of Lorestan province, located between the valleys of Zagros Mountains in the west of Iran, bordering the provinces of Markazi, Hamedan, Kermanshah, Khuzestan, Ilam, and Isfahan. Lorestan covers an area of 28,294 km² with a population of approximately 2 million people. Blood sample (5 mL) was obtained from each patient by venipuncture under sterile conditions. The samples were centrifuged at 1,000 rpm and the sera were stored at –20°C until serological examination.⁷

Questionnaire

Before collection of blood samples, a questionnaire was completed by parents of the children including demographic data (e.g., age, gender), possible risk factors (e.g., animal contacts [dog], and residence), and hypereosinophilia < 10%, one of the main laboratory features of toxocariasis.

Enzyme-Linked Immunosorbent Assay

To measure the anti-*T. canis* antibodies, serum samples were transported to the Parasitology Laboratory, Department of Parasitology and Mycology, Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences (Khorramabad, Iran) and stored at –20°C until tested. All serum samples were tested using the standard commercially available anti IgG-*Toxocara* kit (IBL, Germany) according to manufacturer's instructions. The reaction cutoff was calculated as the mean

optical density (OD) for negative control sera plus three standard deviations (SDs). The positive and negative control sera present in the kit were included in each plate. The reading was acquired using a microplate reader (BioTek, United States) set at a level of absorbance of 450 nm. All samples were run in triplicate. The results were considered positive when OD₄₅₀ index was equal to or higher than the cutoff value in ELISA.

Statistical Analysis

In this study, the analytical and descriptive statistics were performed using SPSS 24.0 software (SPSS Inc., Chicago, Illinois, United States). Descriptive statistics were showed in terms of percentage (for categorical) and mean (SD, for continuous) variables. The chi-square test was applied to assess the univariate association between independent variables and outcome. All the variables in univariate analysis (chi-square test) that had a *p*-value less than 0.25 were entered into multivariate analysis (logistic regression). Multifactorial logistic regression models were used to evaluate association between *T. canis* seropositivity and the potential risk factors. A *p*-value < 0.05 was considered to be statistically significant.

Results

Participants

A total of 316 children (aged 2–15 years) referred to the health centers of Lorestan province, Iran were included in the present study. The mean age of the participants was 7.9 ± 2.5 years and most participants were boys (176, 55.7%). In total, 240 participants (75.9%) lived in urban regions, while the remaining (24.1%) lived in rural areas. Among the participants, 85 children (26.9%) were in contact with dogs, whereas 231 children (73.1%) did not have any contact with dogs. Moreover, only 32 children (10.1%) consumed raw or unwashed vegetables and fruits. Among the children, 299 (94.6%) had no hypereosinophilia while 17 (5.4%) had hypereosinophilia >10% (– Table 1).

Seroprevalence and Risk Factors of *T. canis*

Of the 316 children, 14 (4.4%) tested seropositive for anti-*T. canis* IgG antibody. Seroprevalence of anti-*T. canis* antibody was higher among boys. Ten (5.7%) boys were positive in terms of IgG anti-*T. canis* antibodies compared with four (2.85%) girls (*p* = 0.22). Additionally, a chi-square test for trend revealed that the seroprevalence of anti-*T. canis* IgG antibody did not vary with age among the children between 2 to 15 years (*p* = 0.34).

Out of the 240 participants living in urban regions, 5 (2.1%) tested seropositive for anti-*T. canis* antibodies, whereas from 76 participants who lived in rural areas, 9 (11.9%) tested seropositive for anti-*T. canis* antibodies. There was a significant difference in the prevalence of anti-*T. canis* antibodies among those living in urban and those living in rural areas (*p* < 0.001).

Out of 85 children who had contact with dogs, 10 (11.8%) tested seropositive for anti-*T. canis* antibodies, whereas out

Table 1 Demographic characteristics and seroprevalence of anti-*T. canis* antibodies among children (aged 2–15 years) referred to health centers of Lorestan province, Iran

Variables	No. (%)	IgG positive	p-Value
Gender			
Male	176 (55.7)	10 (6.25)	0.22
Female	140 (44.3)	4 (2.85)	–
Age groups			
< 7y	105 (33.2)	3 (2.9)	–
≥ 7y	211 (66.8)	11(5.2)	0.34
Residential place			
Urban	240 (75.9)	5 (2.1)	–
Rural	97 (20.1)	9 (11.9) ^a	<0.001 ^a
Being in contact with dogs			
No	231 (73.1)	4 (1.73)	<0.001 ^a
Yes	85 (26.9)	10 (11.8) ^a	–
Unwashed vegetables/fruits consumption			
No	284 (89.9)	13 (4.6)	0.70
Yes	32 (10.1)	1 (3.1)	–
Hypereosinophilia (>10%)			
No	299 (94.6)	13 (4.3)	–
Yes	17 (5.4)	1 (5.9)	0.81

^a $p < 0.05$, difference is statistically significant.

of 231 children who had no contact with dogs, 4 (1.73%) tested seropositive for anti-*T. canis* antibodies. There was a significant difference in the prevalence of anti-*T. canis* antibodies among children who had contact with dogs and those with no contact with dogs ($p < 0.001$). Of the 32 children who consumed raw or unwashed vegetables and fruits, 1 (3.1%) was seropositive for anti-*T. canis* antibodies, while out of 284 children who did not eat raw or unwashed vegetables and fruits, 13 (4.6%) tested seropositive for anti-

T. canis antibodies. There was no significant difference in the prevalence of anti-*T. canis* antibodies among the children who consumed raw or unwashed vegetables and fruits and those who did not eat raw or unwashed vegetables and fruits ($p = 0.70$).

Out of 299 children who had no hypereosinophilia, 13 (4.3%) children were positive for anti-*T. canis* antibodies, whereas out of 17 children who had hypereosinophilia > 10%, only 1 child (5.9%) was positive for anti-*T. canis* antibodies.

Table 2 Logistic regression analysis of potential factors associated with anti-*T. canis* antibodies among children (aged 2–15 years) referred to health centers of Lorestan province, Iran

Variables	OR (95% CI)	p-Value
Gender		
Male	2.3 (0.7–8.2)	0.18
Female	1	–
Residential place		
Urban	1	–
Rural	4.2 (1.3–13.9)	0.018 ^a
Being in contact with dogs		
No	1	–
Yes	7.9 (2.3–27.3)	0.001 ^a

Abbreviations: CI, confidence interval; OR, odds ratio.

^a $p < 0.05$ was statistically significant.

There was no significant difference in the prevalence of anti-*T. canis* antibodies among children with no hypereosinophilia and those with hypereosinophilia > 10% ($p = 0.83$).

– **Table 2** shows the association between risk factors and status of anti-*T. canis* IgG antibodies in the logistic regression analysis. The variables used to evaluate association between risk factors and status of anti-*T. canis* IgG antibodies were age, gender, area of residence, contact with dogs, and consumption of raw or unwashed vegetables and fruits. Some risk factors that were significantly related to *T. canis* seropositivity included living in rural regions ($p = 0.018$) and contact with dogs ($p = 0.001$). However, other demographic and risk factors did not demonstrate any association with *T. canis* seropositivity.

Discussion

Toxocariasis as a zoonotic infection is a highly widespread parasitic disease in the tropical and subtropical regions around the world.⁷ Humans can generally be infected by ingesting food and water contaminated with embryonated eggs excreted from feces of a definitive host. In humans, the clinical symptoms of disease vary from asymptomatic illness to severe signs, such as ocular, neurological, and/or systemic involvements, a condition known as visceral larva migrans. The diagnosis of human toxocariasis is typically based on serological tests such as ELISA to detect anti-*Toxocara* antibodies.^{1–3} Because treatments are often only partially effective and no safe and effective vaccines exist, it is critical to make efforts to decrease transmission of toxocariasis to reduce the serious manifestations of disease.⁵ According to review studies, the seroprevalence rate of toxocariasis varies from 2 to 90% in different geographic regions around the world.⁷ Recently, according to a review study conducted by Abdi et al in Iran, the seroprevalence of human toxocariasis, soil contamination with *Toxocara* spp. eggs, and infections in dogs/cats were reported to be 15.8, 21.6, and 26.8%, respectively.⁸ Here, we evaluated the seroprevalence and risk factors for *T. canis* infection in children (2–15 years old) referred to health centers of Lorestan province, Iran. Out of the 316 children, 14 (4.4%) tested seropositive for anti-*T. canis* IgG antibody.

The seroprevalence of *T. canis* infection was less than that reported among children of Jordon,⁹ Netherlands,¹⁰ Turkey,¹¹ Serbia,¹² and Northeast Brazil.¹³ In Iran, several studies have been performed on the seroprevalence of *T. canis* infection among children including the study conducted by Sadjjadi et al;¹⁴ the total prevalence of anti-*Toxocara* antibodies in the sera of school children of Shiraz, southern Iran was 25.6%. Akhlaghi et al¹⁵ have reported the seroprevalence of anti-*Toxocara* antibodies to be 8.6% among children (2–15 years old) in Kermanshah province, Iran. In another study, Ghaffar-Naqnehi et al¹⁶ found the seroprevalence of *T. canis* infection in children 2 to 14 years old referred to health care centers of Chaharmahal and Bakhtiari province, Iran to be only 2%. Moreover, in the study of Alavi et al,¹⁷ the seroprevalence rate of *T. canis* was 2% in rural and urban school children aged 6 to 15 years in Ahvaz, Iran. Fallah et al¹⁸ found that 29 out of 544 children were positive for

anti-*Toxocara* antibodies showing an overall prevalence of 5.3% in the western Islamic Republic of Iran. These variations in the prevalence of anti-*Toxocara* antibodies among children in various parts of Iran and the world might be related to sample size, method of testing, transmission routes, and climatic conditions of the studied region and country.¹⁷

In this study, similar to the work conducted by Sadjjadi et al,¹⁴ we found a significant difference in the prevalence of anti-*T. canis* antibodies among children living in urban and rural areas ($p < 0.01$); however, it was in contrast to the findings of Fallah et al¹⁸ and Alavi et al.¹⁷ These variations might be due to work-related activities associated with contact with dogs or having a less hygienic lifestyle. Here, we found that having contact with dogs is a significant risk factor for *T. canis* seropositivity. Similarly, Ghaffar-Naqnehi et al¹⁶ and Alonso et al¹⁹ demonstrated that contact with dogs is a risk factor for *T. canis* infection. In the present study, no significant difference was found in age, gender, consumption of raw or unwashed vegetables and fruits, and *T. canis* seropositivity. In line with these results, Alonso et al¹⁹ and Fallah et al¹⁸ found no significant difference in terms of age, sex, and consumption of unwashed vegetables and fruits. Here, we found that there was no significant difference in the prevalence of anti-*T. canis* antibodies among children with no hypereosinophilia and those with hypereosinophilia > 10%. In line with our results, some studies in Brazil and Ahvaz also did not demonstrate a significant association between eosinophilia and toxocariasis.^{17,20,21} However, in a study by Alonso et al in Argentina, a significant connection was found between eosinophilia and toxocariasis.¹⁹

These differences in the seroprevalence of anti-*Toxocara* antibodies among children could be related to sociocultural behaviors, geographical and environmental parameters, and transmission routes in the populations.^{17,22}

Conclusion

We found that *T. canis* infection is prevalent among children (2–15 years old) referred to health centers of Lorestan province, Iran with an overall seroprevalence rate of 4.4%. These findings may be a warning for health centers to pay special attention to toxocariasis among children (2–15 years old) and design screening programs for the prevention of toxocariasis.

Conflict of Interest

The authors declare that there is no conflict of interest.

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