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Toxocara Seroprevalence and Associated Risk Factors Among Ilam Children, West of Iran

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Abstract

Background: Human toxocariasis is caused by *Toxocara canis* and *cati*, which are the main ascarids of dogs and cats, respectively. Most infected humans are asymptomatic and our understanding of human disease burden is limited by lack of epidemiological studies and insufficient clinical awareness.
Objectives: There is no precise report on the seroprevalence of toxocariasis in Ilam children. Therefore, this study found an opportunity to investigate this subject.
Methods: In this cross-sectional study, 300 sera of children aged between 2 to 15 years old were collected during March 2016 to February 2017 in urban and rural areas. Demographic variables were filled for each person in accordance with risk factors along with sampling. Some hematological parameters were measured. The sera were examined for anti *Toxocara canis* and *cati* antibodies, according to the ELISA kit protocol.
Results: Among a total of 300 serum samples, 35 (11.7%) were positive for anti-*Toxocara* IgG. The infection rates were 5.3% and 6.3% in female and male, respectively. There was no significant relationship between gender and *Toxocara* infection rates (P = 0.59). A total of 26 (17.3%) of the 150 rural children, and nine (6.0%) of the 150 urban children were positive. There was a significant relationship between place of living with ELISA tests results (P = 0.004). Hematologic parameters showed a significant increase in the numbers of peripheral eosinophil in the sample of patients whose sera were positive (P = 0.037).
Conclusions: High prevalence of toxocariasis among Ilam children in the west of Iran can be considered as a public health problem. The evaluating infection control programs in dog and cats are necessary for controlling the disease in this region.

Keywords: *Toxocara*, Seroprevalence, Children, Iran

1. Background

The genus *Toxocara*, including *Toxocara canis* and *T. cati*, are recognized as causative agents of human toxocariasis. Definitive hosts of these common nematode parasites are dogs and cats (1). The unembryonated eggs are excreted in feces by the adult animal and become infectious under appropriate environment conditions. Humans are infected by ingestion of embryonated eggs. In small intestine larvae hatches, it penetrates the intestine mucosa and migrate via bloodstream to the liver, eyes, lungs, muscles, and central nervous system. *Toxocara* larvae cannot develop to adult worms in humans (2).

Most cases of human toxocariasis are asymptomatic infection. Considering different symptoms of disease, four syndromes are recognized: Visceral larva migrans, ocular larva migrans, neurological toxocariasis (NLM), and covert toxocariasis (3).

Overall prevalence of toxocariasis has been determined in Iran with a growing trend of 21.6% and contributions of seropositivity for human, dog, and cat infections; adult, worm, and soil contamination with eggs are 15.8%, 26.8%, and 21.6%, respectively (4). In developed countries, it has low seroprevalence rates of *Toxocara* infection, for example 0.7 % in New Zealand and 1.6 % in Japan (5, 6). In-

terestingly, higher seroprevalence rate has been reported with 13.9% in USA (7). It shows that toxocariasis is a public health problem even in developed countries.

The standard immunodiagnostic test of human toxocariasis is enzyme-linked immunosorbent assay (ELISA) using *T. canis* excretory-secretory (TES) antigens. This method has been reported to be 78% sensitive and 92% specific (8-10). Infections with the *Toxocara* occur widely around the world and are more prevalent in children. Being young, low levels of parental education, low socioeconomic status, poor sanitation, playing in sandpits, and pica are factors contributing to *Toxocara* exposure (11-13). Although most infected humans are asymptomatic and our understanding of the burden of human disease, particularly children, is limited by lack of epidemiological studies and insufficient clinical awareness, improved understanding of this common neglected infection would assist its control (4).

2. Objectives

To the best of our knowledge, there is no precise report from the seroprevalence of toxocariasis in Ilam children. Therefore, this study afforded an opportunity to investigate this subject.

3. Methods

3.1. Ethics Statement

The current study was approved by the Ethics Committee of Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran (IR.THUMS.REC.1396.63).

3.2. Sample Size Calculation

Like a similar study (14), the sample size was calculated on seroprevalence of 30%, d = 0.052 at a confidence level of 95%. The study population size was obtained to be 298.

3.3. Study Population and Questionnaire Interview to Assess Risk Factors

In this cross-sectional study, 300 sera of children (150 girls and 150 boys) aged between two to 15 years old were randomly collected during March 2016 to February 2017 in urban and rural areas of Ilam. Age and gender ratios were kept constant between the regions. All procedures were in accordance with the ethical standards of responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008. Informed consent forms were obtained from the parents for the children and the purpose and procedures of the studies were explained to all participants and their parents. Demographic variables, in accordance with risk factors including age, sex, onychophagy, geophagy and keeping dogs and/or cats, was checked for each person along with sampling. No clinical sign from children was checked.

3.4. Serum Samples

After collecting about 3 mL of the whole blood samples in tubes, serums were separated and stored at -20°C until analyzed.

3.5. Stool Examination and Hematological Parameters

In order to prevent cross-reactions between *Toxocara* larvae and other organism's antigens, stool exam was performed for the seropositive cases using wet mount and formalin-ethyl acetate technique (15). In addition, patients who were infected with the other parasites, especially Ascarididae family, were excluded. We measured some hematological parameters such as ESR values and WBC count.

3.6. ELISA

All sera were examined by anti-*Toxocara* IgG-ELISA test with NOVATEC kit (GMBH, Germany), according to kit protocol. An absorbance reading greater than 0.5 optical density (OD) units was considered as a cut off for seropositivity. Positive and negative controls were also used in each run.

3.6. Statistical Analysis

Results were analyzed with SPSS version 16.0 (SPSS Inc. Chicago, Illinois). Data for host factors and risk factors were examined using Pearson Chi-square and Fisher's exact tests analysis. P values < 0.05 were considered significant. The relative proportions were calculated with confidence intervals (CI) of 95%.

4. Results

From the total of 300 serum samples, 35 (11.7%) were positive for anti-*Toxocara* IgG using ELISA technique. The infection rates in female and male were 5.3% and 6.3%, respectively. There was no significant relationship between gender and *Toxocara* infection rates (P = 0.59).

A total of 26 (17.3%) of the 150 rural children, and nine (6.0%) of 150 urban children were positive for anti-*Toxocara* IgG antibodies. There was a significant relationship between place of living with ELISA tests results (P = 0.004).

In this study, 4%, 0.7%, 29.3%, and 12.7% of the seropositive cases had onychophagy, geophagy, soil contact, and dog contact history, respectively. No variables were significant in the analysis for these risk factors related to the seropositive tests ($P > 0.05$) (Table 1).

Hematologic parameters in seronegative and seropositive cases were described and compared (Table 2). Quantitative analysis showed a significant increase in the numbers of peripheral eosinophil, which were counted using the prepared blood smears, in the sample of patients

Table 1. The Relationship Between Associated Risk Factors with the *Toxocara* Seroprevalence Among Ilam Children

Variables	Seropositive, No. (%)	Seronegative, No. (%)	Odds Ratio (OR)	95% CI Lower-Upper	P Value
Age group, y			-	-	0.058
0 - 5	5 (1.7)	24 (8.0)			
5 -10	10 (3.3)	125 (41.7)			
10 - 15	20 (6.7)	116 (38.7)			
Sex			1.215	0.599 - 2.464	0.59
Male	19 (12.6)	131 (87.4)			
Female	16 (10.6)	134 (89.4)			
Residency			3.28	1.483 - 7.278	0.004
Rural	26 (17.3)	124 (82.7)			
Urban	9 (6)	141 (94)			
Contact with soil			0.805	0.37 - 1.749	0.58
Yes	10 (10.2)	88 (89.8)			
No	25 (12.4)	177 (87.6)			
Contact with dog/cat			2.321	0.967 - 5.572	0.06
Yes	8 (21.1)	30 (78.9)			
No	27 (10.3)	235 (89.7)			
Geophagy			-	-	1.00
Yes	0	2 (100)			
No	35 (11.7)	263 (88.3)			
Onychophagy			2.667	0.686 - 10.362	0.15
Yes	3 (25)	9 (75)			
No	32 (11.1)	256 (88.9)			

whose serum was positive for anti-*Toxocara* antibodies (P = 0.037). There was no significant difference in other parameters (P > 0.05).

5. Discussion

The seroprevalence of *Toxocara* in two to 15 year old children from Ilam was examined with anti-*Toxocara* IgG-ELISA test and estimated to be 11.7%.

Different seroprevalence of toxocariasis was reported in various countries to be 2.4% in Denmark (16) to 92.8% in La Reunion (17).

In this study, the seropositive rates for *Toxocara* in children was high compared to other parts of Iran, such as 2% in Isfahan and 5.2% in Chaharmahal and Bakhtiari province (central) (18, 19), 1.39% in Ahvaz (southwest) (20), 8.8% in Hamadan and 8.46% in Kermanshah province (west) (21, 22), as well as 2.7% in Zanjan city and 3% in Urmia (northwest) (23, 24). However, it was relatively low compared to 25.6% in Shiraz province (southern) (12), 25% in Sari city (north) (14), and 29.46 % in East Azerbaijan province (northwest) (25).

Difference in climate, culture, religion, life style factors, variation of detection methods, and lack of standardization of clinical disease definitions could explain some reasons of *Toxocara* seroprevalence of different population (26).

Some large-scale studies have shown no association between gender and toxocariasis (12, 20, 27). In our study, there was no significant correlation between gender and seropositivity (P = 0.58). In addition, 26 infected children were living in rural areas and nine in urban areas; the correlation between places of living with seropositivity was statistically significant (P = 0.004).

Similar to our finding, Sadjjadi et al. (12), reported a significant correlation between places of living with seropositivity. However, in contrast to our finding; Alavi et al. (20), did not find a statistically significant correlation. The high prevalence of toxocariasis in the studies may be the result of more contact with infected dogs and cats in rural regions.

Dog/cat contact has been recognized as a risk factor in several studies (28-30). Although the infection rate in this study is higher among those who had contact with dog/cat

Table 2. The Relationship Between Hematologic Parameters with the *Toxocara* Seroprevalence Among Ilam Children.

Hematologic Parameter	Seropositive, No. (%)	Seronegative, No. (%)	Odds Ratio (OR)	95% CI Lower-Upper	P Value
Leukocytosis			1.54	0.324 - 7.361	0.63
Yes	2 (16.7)	10 (83.3)			
No	33 (11.5)	255 (88.5)			
Neutrophilia			1.754	0.621 - 4.956	0.34
Yes	5 (17.9)	23 (82.1)			
No	30 (11)	242 (89)			
Lymphocytosis			0.663	0.324 - 1.357	0.25
Yes	20 (10.2)	177 (89.8)			
No	15 (14.6)	88 (85.4)			
Eosinophilia			16.0	1.412 - 181.303	0.037
Yes	2 (66.7)	1 (33.3)			
No	33 (11.1)	264 (88.9)			
ESR			2.331	0.301 - 18.075	0.7
Normal	34 (12.1)	248 (77.9)			
Abnormal	1 (5.6)	17 (94.4)			

than others, this difference does not have analytical value and significance (P = 0.06). Moreover, there was no statistically significant correlation between other behavioral factors; i.e. soil contact, geophagy, onychophagy, and seropositivity (P > 0.05).

According to the results, seropositive cases had significantly higher eosinophilia in comparison with seronegative cases (P = 0.037), however, hypereosinophilia was not observed in any of the seropositive cases. The data are compatible with other data for the epidemiology of toxocariasis in eosinophilic patients (24, 31). Cross-reactive antibodies elicited by exposure to other helminths may reduce the specificity of ES antigen for the diagnosis (32). Therefore, we examined the stool specimen of the seropositive individuals for the helminthic contamination by the formalin-ethyl acetate technique, which is a strong point of our study. The main limitations of this study were lack of checking on the clinical sign and not being able to conduct a follow up on the infected children.

5.1. Conclusions

This study helps us increase the awareness about *Toxocara* infection among Ilam children. The high prevalence of *Toxocara* infection in children in the west of Iran can be considered as a public health problem and evaluating infection control programs in dogs and cats are necessary for control of toxocariasis in this region. We recommend that children with eosinophilia of an unknown origin are evaluated for possible *Toxocara* infection. This evaluation would prevent misdiagnosis of idiopathic eosinophilia.

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Footnotes

Authors' Contribution: Mohammad Ali Mohaghegh, Saleh Khoshnood, Nader Pestehchian, Seyed Hossein Hejazi, and Abdollah Rafiei: Conceptualized the study and developed search strategy; Ali Soleimani, Mahmoud Ahmadi, and Saleh Khoshnood: Collection of samples; Mohammad Ali Mohaghegh, Saleh Khoshnood, Nader Pestehchian, and Zahra Jabalameli: Execution techniques and parasitological examination; Mohammad Ali Mohaghegh, Abdollah Rafiei, and Saleh Khoshnood: Analysis and interpretation of data; All authors reviewed and contributed to the writing of this manuscript.

Conflict of Interests: The authors declare that they have no conflict of interest.

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