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### Original Article

## Seroprevalence of Human Toxocariasis in Children (5–15-Year-Old) Using ELISA Method in Ardabil District, North-West of Iran

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### **Abstract**

**Background:** Toxocariasis is a worldwide-distributed helminth parasitic infection. This study aimed to evaluate the seroprevalence of human toxocariasis in children living in Ardabil Province, North-West of Iran.

**Methods:** A seroepidemiological study to evaluate human toxocariasis among urban and rural populations of Ardabil County, North-West of Iran using ELISA test was carried out from 2019 to 2020. The study population was 472 children (185 females and 287 males) aged between 5 and 15 yr old. Immunoglobulin G antibodies against *Toxocara* spp. were analyzed by ELISA test.

**Results:** Of the 472 collected serum samples, 66 (14.0%) were positive for antibodies against human toxocariasis. The highest prevalence of infection was observed in children with age groups of 5–6 years. Data demonstrate higher seroprevalence among males (15.3%) than females (11.8%). Rural areas prevalence was significantly higher (24.4%) than the urban area (8.65%). The rate of the diseases was 22.8% in children having history of contact with dog.

**Conclusion:** Toxocariasis is prevalent in the children of Ardabil region. The present study can increase the awareness of the population about the risk of zoonotic diseases as well as the anthelmintic treatment of dogs by veterinarians and, more importantly, the control of stray dogs.



## Introduction

**T**oxocariasis is a worldwide-distributed helminth parasitic infection caused by the parasitic roundworms that usually lives in the small intestine of dogs (*Toxocara canis*) and cats (*T. cati*) (1). Humans become infected by ingestion of embryonated *T. canis* and *T. cati* eggs or raw meat/viscera of infected paratenic hosts (2). The eggs hatch and larvae can be distributed into all organs such as liver, lungs, brain and eyes by blood circulation. Infected eggs can be ingested by humans as an accidental host, in which case the eggs hatch in the intestine, the larvae are released and penetrate the intestinal wall, and through the intestinal blood and lymph vessels to the liver, brain, the eyes and other tissues migrate, where they remain metabolically active as migrating larvae for months without growth or differentiation (3). The larvae can survive in tissue for several years.

They can cause severe local reactions, which is a major problem in toxocariasis. The wandering of the second-stage larvae among the tissues is characterized by hemorrhage, necrosis, infiltration of mononuclear cells and eosinophils. A granuloma forms around the larvae that separate them from the host tissues. The variety of signs and symptoms of the disease depends on the number of ingested embryonic eggs, the number and location of granulomas, and the host's allergic responses to the larval antigen. Three clinical forms are classified as visceral larva migrans (VLM), ocular larva migrans (OLM), and common, neurologic, and covert toxocariasis (4).

Mild infections are usually asymptomatic and the only manifestations are peripheral eosinophilia (20%- 40%) and mild fever of unknown cause (Fever of unknown origin=FUO) (5). In severe infections, eosinophilia is up to 90% (6). Human toxocariasis is contained in the list of neglected diseases. Seropre-

valence assessments in different countries have shown the global distribution of toxocariasis (1).

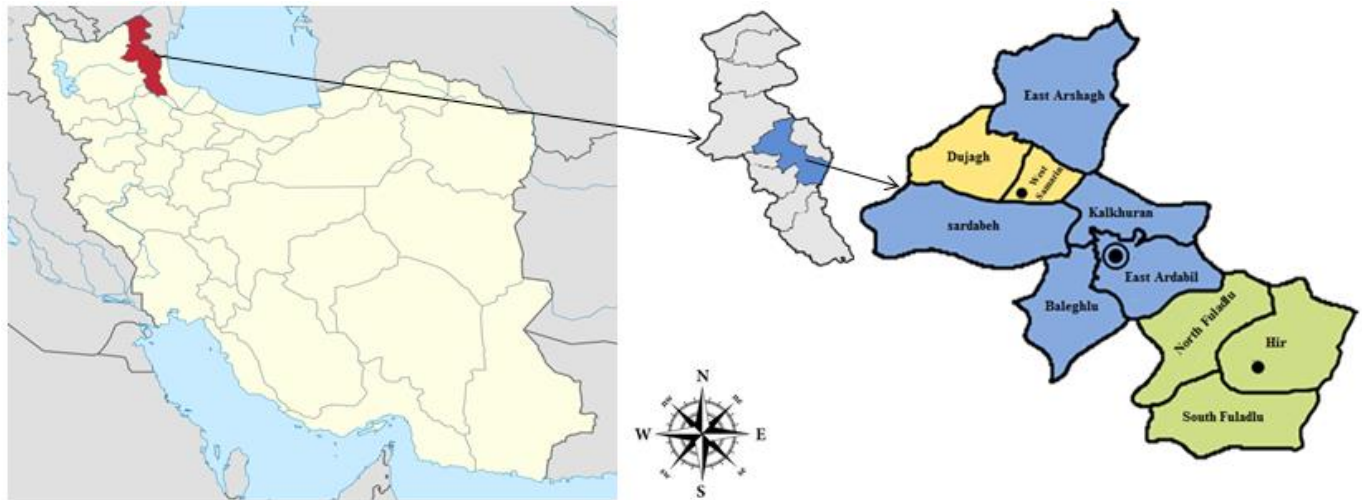
Due to the relatively high prevalence of *Toxocara* parasite among dogs and cats in Iran, the need to pay attention to the disease caused by this parasite (toxocariasis) is felt as a potential medical and health risk in the country. *Toxocara* infection rate in Iran is estimated 9.3% (7, 8). Serological techniques are reliable methods for detecting larval antigen antibodies. The best choice for serodiagnosis of toxocariasis is the ELISA test using parasite-secreting antigens (*T. canis* excretory secretory Ag (TES-Ag) (9).

Ardabil City has a high density of stray dogs and cats. Due to there is no previous report about the seroprevalence of human toxocariasis in Ardabil City, the present study was carried out to estimate the seroprevalence of toxocariasis in different regions of Ardabil City, Iran using the ELISA test. Moreover, its relationship with epidemiological variables was determined.

## Materials and Methods

### Study Area

Ardabil Province is located between 38.2514 °N and 48.2973 °E in the northwest of Iran. This province is bounded on the north by the Republic of Azerbaijan, on the west by the province of East Azerbaijan, on the east by the province of Gilan, the Talesh Mountains, and on the south by the province of Zanjan. The estimated population of Ardabil is about 1,270,420 million (2016). The province is divided into 10 counties: Ardabil (capital), Bilasavar, Germe, Khalkhal, Kowsar, Meshginshahr, Namin, Sarein, Nir, and Parsabad (Fig.1).



**Fig. 1:** Left: map of Iran and Ardabil Province, Right: map of districts in Ardabil Province, from where the serum samples were collected

### **Samples**

The current cross-sectional study was conducted on 472 sera of children (185 women and 287 men) aged between 5 and 15 yr old randomly selected from different districts of the Ardabil County in 2019-2020. A short questionnaire to obtain data concerning their age, gender, residency, and contact with dogs and eating uncooked vegetable-filled for each child by the parents. For evaluation of previous contact to *Toxocara*, blood samples were collected from all individuals then sera were separated and stored in -20 °C until tested.

The study was approved by the Ethical Committee of Ardabil University of Medical Sciences (IR.ARUMS.REC.1398.116).

### **ELISA test**

The diagnostic test of *Toxocara* antibody (IgG) was performed in all serum samples using commercial *Toxocara* IgG ELISA kit (NovaTec, Germany) according to the manufacturer's recommendations, and each test was read independently by 2 different operators. The optical density (OD) value was recorded in an automatic ELISA reader at 450 nm.

### **Statistical analysis**

All data were analyzed using SPSS software ver. 22 (IBM Corp., Armonk, NY, USA) and Chi-square test. Differences were considered significant if *P*-values were less than 0.05.

### **Results**

Cut-off value was calculated 0.43. Of the 472 collected serum samples, 66 (14%) were positive for antibodies against human toxocariasis (Fig. 2). The highest prevalence of infection was observed in children with age groups of 5-6 yr and statistically significant differences ( $P= 0.000$ ). Other age group's infectivity is obvious in Fig. 3. Data from this study demonstrate higher seroprevalence among males (15.3%) than females (11.8%) ( $P= 0.000$ ). Rural areas prevalence was significantly higher (24.4%) than the urban area (8.65%) ( $P= 0.000$ ). The rate of the diseases was 22.8% in children having history of contact with dog ( $P= 0.000$ ). No statistically significant differences were seen between seropositivity to *Toxocara* infection and eating uncooked vegetables (Table 1).

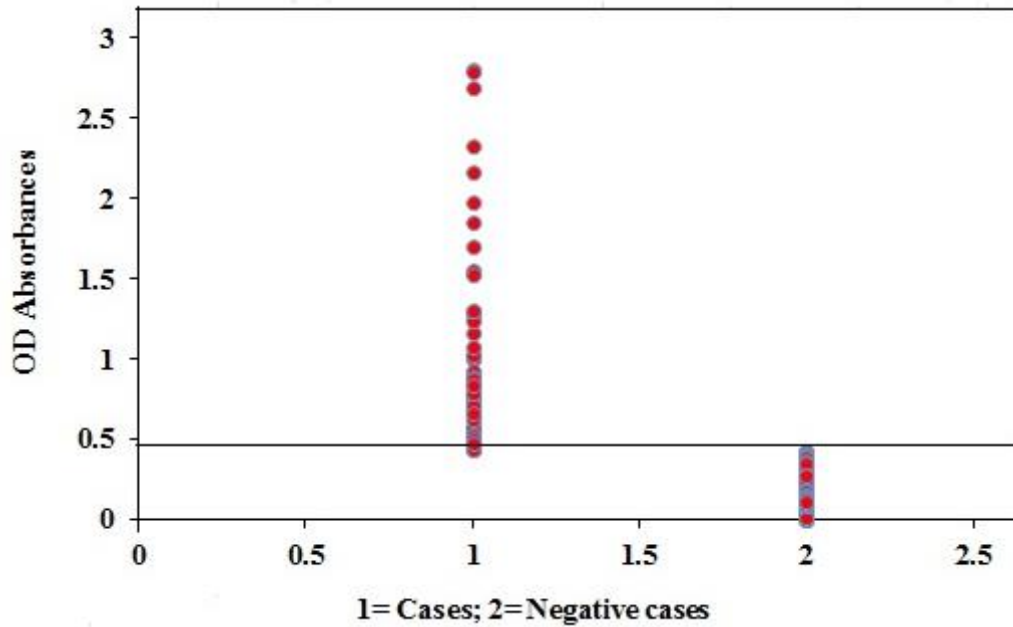


Fig. 2: Analysis of sera from subjects and normal controls by IgG-ELISA

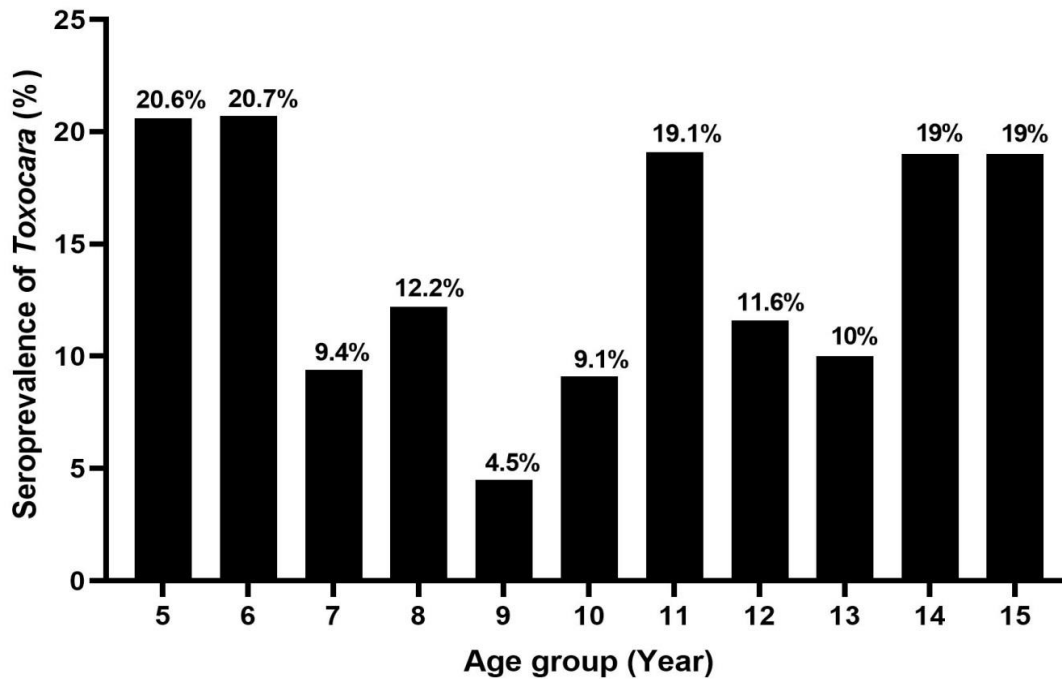


Fig. 3: Distribution of positive cases of toxocariasis using ELISA according to age group (yr) in Ardabil Province, North-West of Iran

**Table 1:** The relationship between gender, location, contact with dogs, eat raw vegetable with the ELISA test result in children aged 5–15 yr old that were randomly selected from different districts of the Ardabil county in 2019-2020

<i>Risk factor</i>	<i>No. (%) of analyzed samples</i>	<i>No. (%) of positive</i>	<i>No. (%) of negative</i>	<i>P-value</i>
Sex				<i>P</i> = 0.000
Male	294 (62.3)	45 (15.3)	249 (84.6)	
Female	178 (37.7)	21 (11.8)	157 (88.2)	
Residency				<i>P</i> = 0.000
Rural	160 (33.9)	39 (24.4)	121 (75.6)	
Urban	312 (66.1)	27 (8.65)	285 (91.3)	
Contact with dogs				<i>P</i> = 0.000
Yes	158 (33.47)	36 (22.8)	122 (77.2)	
No	314 (66.52)	30 (9.55)	284 (90.4)	
eating uncooked vegetable				<i>P</i> > 0.05
Yes	206 (43.6)	27 (13.1)	179 (86.8)	
No	266 (56.35)	39 (14.6)	227 (85.3)	

## Discussion

The global prevalence of *Toxocara* in humans is influenced by some individual factors, including climatic, health, economic and cultural conditions (1). A large number of studies have been demonstrated that VLM may be the second most common parasitic infection (10, 11). The prevalence of this disease varies in different countries. The overall prevalence of toxocariasis in Iran is 21.6% (12). A systematic review and meta-analysis study reported that the weighted mean prevalence of human cases with overall 28 records was 9.3%. The percentage prevalence of *Toxocara/Toxascaris* in dogs and cats was 24.2% and 32.6%, respectively (7). The high seroprevalence of toxocariasis in the Iranian population was observed in systematic review study (13).

Currently, there are no previous published reports of human toxocariasis prevalence in Ardabil Province, North-West of Iran. To our knowledge, this is the first epidemiological survey on seroprevalence of human toxocariasis in Ardabil City using ELISA method. In the present study, 67 (14.1%) of 472 individu-

als were seropositive for toxocariasis. Hosseini-Safa et al. demonstrated that 6 (1.39%) of 427 children were seropositive for toxocariasis in Isfahan Province (14). 7.2% of the study population has Anti-*Toxocara* antibodies in northeastern Iran (15). Recently, of the 200 collected soil samples the eggs of *Toxocara* spp. were detected in 14 (7%) soil samples. These results confirm the hypothesis that the rate of infection is probably due to the high frequency of the dogs and cats in Ardabil (16).

The results obtained in the present study are consistent in some cases and different in some compared to some similar populations and age groups. In this study, the highest prevalence of infection was observed in children with age groups of 5-6 years. In Ahwaz, Iran, 56.2% seropositivity were reported among individuals aged 6–8 yr old (17). A prevalence of 37.4% for toxocariasis was reported among 0–5 yr old children in Fars Province, southern Iran (18). In Shiraz, among children between 6-13 yr ages, the prevalence rate was reported to be 15%.

Besides, 20.2% and 30.1% were allocated to rural and urban areas, respectively.



In the present study, higher seroprevalence among male (68%) than females was observed. From 427 children, 196 (45.9%) were female and 231(54.1%) were male (14). Male children were more infected (41.71% as compared to females (24.16%) (19).

However, in an epidemiologic study, seroprevalence of 19% (n= 19) was reported for toxocariasis in hypereosinophilic individuals (20). Probably the reason for the difference is the play games and the specific behavior of the people. The study of the relationship between the prevalence of toxocariasis and host factors including age and sex showed that there was no significant difference between the age groups of 2-6 yr and 7-12 (21).

The reason for the relationship between age and the prevalence of toxocariasis in children, especially those aged 1-3 yr, has been mentioned in many evaluations as soil-eating and ingestion of substances contaminated with parasite eggs. However, some areas with high prevalence of toxocariasis infection in humans did not show a significant difference based on age (10, 22). In Trinidad, among primary school children and in Argentina among children aged 1-14 yr, no significant difference was observed between age and the presence of anti-*Toxocara* antibodies (23, 24). While in Jordan, significant differences were reported between different age groups (25). Moreover, in Shiraz, this relationship was not observed (21).

24.4% of rural and 8.65% of urban individuals in Ardabil Province was infected with toxocariasis. The results obtained in the urban areas of Shiraz are contrary to other reports, justified by the high level of infection of cats and dogs with *T. canis* and *T. cati* in this city (21). 56.2% of rural and 43.8% of urban children in Ahwaz Province were infected with toxocariasis (17). Moreover, four of infected children were from urban (1.25%) and two from rural areas (1.9%) (14). *Toxocara* seroprevalence in rural and urban areas of northeastern Iran was 9.6 and 4.1% respectively (15).

In addition, in the present study, the prevalence of toxocariasis in children having a history of contact with dogs is high (22.8%) and there is a significant correlation between contact with dog, and seroprevalence of toxocariasis. In a case-control study, seroprevalence of toxocariasis in cat and dog owners are very high (20.43%) compare with other people (1.07%) in Mashhad, Northeastern Iran (26). The highest rate of toxocariasis was observed in children having contact with dogs (45.07%) or with pets (60.71%) in East-Azerbaijan Province, Iran (19). Contact with dogs or cats were one of the risk factors for seropositivity of toxocariasis in northeastern Iran (15). The similar result was shown in Sharif et al. study Northern Iran (27). In systematic review and meta-analysis study, close contact with dogs was one of the main potential risk factors associated with seropositivity to *Toxocara* in people worldwide (28). In some previous studies, no statistically significant correlations between contact with dogs and prevalence of toxocariasis were observed. Keeping the dogs at home as a risk factor for human toxocariasis is debatable. Some authors have reported higher rates of infection for those who have been in contact with dogs, while others have found no association between keeping dogs and toxocariasis; however, contact with dogs is important in human infection with toxocariasis (14, 29-31).

In the current study, no statistically significant differences were seen between seropositivity to *Toxocara* infection and eating uncooked/raw vegetable. There was no significant difference between consumption of raw/unwashed vegetables and *T. canis* seropositivity in children (2–15 yr old) referred to Health Centers of Lorestan Province, Iran (32). Children having history of eating raw vegetable are more infected with *Toxocara* (36.84%) compare with children have not history of eating raw vegetable (25.84%) (19). Statistically significant differences were seen between toxocariasis and eating raw vegetable

(15). Seroprevalence of *Toxocara* spp. in children had a history of eating of raw vegetables is high compare with other group in in Zahedan, Southeast of Iran also there was no significant difference (33). *Toxocara* spp. infection was high in children consuming raw vegetables but significant differences were not seen (34).

## Conclusion

The present study confirms the prevalence of this disease as a public health problem in the study areas. In general, toxocariasis in Ardabil Province can be considered as a public health problem and physicians need to pay attention to this disease and its clinical signs that is closely similar to other diseases to mention their diagnosis, especially in children with chronic eosinophilia, enlarged liver, or non-specific lung disease, toxocariasis. This study can also be effective in increasing the health and public awareness about this disease and in addition to the accuracy of veterinarians in the periodic treatment of intestinal worms in dogs and, more importantly, confirms the need to control stray dogs.

## Acknowledgements

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## Conflict of interest

The authors declare that there is no conflict of interest.

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