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Research Paper

Seroprevalence and Associated Risk Factors of Toxocariasis in Children With Hypereosinophilia in Iran

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ABSTRACT

Introduction: Toxocariasis is a significant parasitic disease with a global distribution, caused by two nematodes: *Toxocara canis* (a parasite of the dog's small intestine) and *Toxocara cati* (a parasite of the cat's small intestine). Eosinophilia, characterized by an elevation in peripheral blood eosinophil counts exceeding 10%, serves as a significant clinical marker for helminthic infections. This study aims to investigate the seroepidemiology of *T. canis* and its risk factors in children with hypereosinophilia in Lorestan Province, Western Iran.

Materials & Methods: This cross-sectional study was conducted from May 2021 to June 2023, involving a cohort of 300 children aged 2 to 15 years who presented with eosinophilia (>10%) and were referred to healthcare facilities in Lorestan Province. Children who were younger than 12 years (between 2-12 years), had blood eosinophil counts exceeding 10%, and consented to participate in this study were deemed to fulfill the inclusion criteria. The prevalence of toxocariasis was confirmed serologically by detecting human IgG antibodies utilizing commercially available anti-IgG-*Toxocara canis* kits. Demographic and some risk factors regarding the enrolled subjects, including gender, age, type of residence, consuming unwashed vegetables and fruits, and history of animal contact, were collected.

Results: Out of a total of 300 children with eosinophilia, 35(11.7%) were identified as seropositive for toxocariasis. Specifically, female participants were less likely to contract toxocariasis than male children [P=0.002, OR=0.184 (0.052-0543)]. Additionally, individuals living in urban environments exhibit a 4.54-fold increased likelihood of contracting toxocariasis compared to their rural counterparts [P=0.010, OR=4.546 (1.433-9.419)]. Furthermore, individuals who did not consume unwashed vegetables demonstrated a reduced risk of toxocariasis infection [P=0.001, OR=0.016 (0.005-0.048)].

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Archives of Razi Institute Journal

Conclusion: The findings of our study indicated a significant seroprevalence of toxocariasis among children exhibiting eosinophilia in western Iran. This suggests that toxocariasis should be regarded as a potential etiological factor for eosinophilia, particularly in individuals with a history of consuming unwashed fruits and vegetables, as well as those residing in rural regions of Iran.

1. Introduction

Toxocariasis is a significant parasitic disease with a global distribution, caused by two nematodes: *Toxocara canis* (a parasite of the dog's small intestine) and *Toxocara cati* (a parasite of the cat's small intestine) [1]. Infection occurs in humans through contact with soil contaminated with animal feces (from dogs, cats, and infected puppies) or by consuming vegetables that have been contaminated with parasite eggs [2]. While the majority of infections in humans remain asymptomatic, the larvae derived from eggs or the larvae themselves can infiltrate multiple organs, resulting in clinical manifestations such as visceral larva migrans and ocular symptoms [3]. Eosinophilia is characterized by an elevation in the levels of eosinophils in peripheral blood, specifically when these levels exceed 10%, and it is a hallmark clinical indicator of helminth infections [4]. Toxocariasis manifests as an immune response elicited by the host in reaction to the migrating larvae of *Toxocara* species [5]. Research has demonstrated that *Toxocara* larvae induce severe eosinophilia by influencing IgE antibodies and IL-5 cytokines, which can lead to allergic complications [6, 7].

In recent years, a significant increase has been observed in the population of dogs and cats in our country, which has consequently led to an increase in soil contamination by *Toxocara* eggs [8]. This development heightens the risk of human exposure to and infection by this parasite, resulting in the potential for toxocariasis [8]. Research conducted in Iran has indicated that the level of soil contamination in parks by this parasite ranges from a minimum of 3.9% in Urmia in the West Azerbaijan Province to a maximum of 63.3% in Khorramabad, Lorestan Province [8]. Based on seroepidemiological studies conducted in Iran, the total frequency of toxocariasis among humans has been established at 11%; whereas the pooled prevalence of *Toxocara* infection was found to be 17%, 37%, 18%, and 2% in dogs, cats, soil, and raw vegetables, respectively [9].

Research indicated that the probability of encountering *Toxocara* infection is markedly increased during the early years of life, particularly within the first decade. This heightened risk can be attributed to various factors, including the geophagic behaviors commonly exhibited by children, their frequent interactions with definitive hosts, and engagement in activities involving soil [10]. A recent study conducted in Lorestan Province, Iran, revealed that out of a total of 316 children aged 2 to 15 years, 14 individuals, representing 4.4%, tested positive for the anti-*Toxocara canis* IgG antibody [11]. Given the notable prevalence of the *Toxocara* parasite in this region, this study aimed to investigate the seroepidemiology of toxocariasis and its risk factors in children with hypereosinophilia in Lorestan Province, Western Iran.

2. Materials and methods

2.1. Ethics

This research received approval from the Ethics Committee of Lorestan University of Medical Sciences, Khorramabad, Iran. Furthermore, written informed consent was secured from the parents of the participating children prior to the collection of blood samples.

2.2. Study design

This cross-sectional study was carried out between May 2021 and June 2023, involving a cohort of 300 children aged 2 to 15 years who presented with eosinophilia and were referred to healthcare facilities in Lorestan Province, western Iran.

2.3. Inclusion and exclusion criteria

Children who were younger than 12 years (between 2-12 years), had blood eosinophil counts exceeding 10% in complete blood count (CBC), and who consented to engage in this study were identified as meeting the inclusion criteria. In contrast, children who had been diagnosed with asthma, chronic sinusitis, atopic dermatitis, or drug allergies within the previous year were excluded, and children with immune system defects as well as those who received antibiotic or antiparasitic drugs were excluded from participation in this research.

2.4. Sample collection

A volume of 5 mL of blood was collected from each participant, following established sterile procedures. The samples were then subjected to centrifugation at 1,000 rpm, and the resultant sera were stored at -20 ˚C until the serological analysis was conducted.

2.5. Questionnaire

A structured questionnaire was administered through interviews conducted by trained nursing professionals, following the acquisition of informed consent from the participants or their parents. Demographic data pertaining to the enrolled participants, encompassing variables such as gender, age, and type of residence, were gathered. The questionnaire encompassed inquiries about the subjects' history and frequency of consuming unwashed vegetables and fruits, along with their historical interactions with animals and experiences with pet ownership, including dogs and cats.

2.6. Enzyme-linked immunosorbent assay (ELISA)

The frequency of toxocariasis was estimated through the identification of human IgG antibodies using an anti-IgG-*Toxocara canis* kit (IBL, Germany), following the manufacturer's specified protocols. Absorbance readings were obtained using a microplate reader (BioTek, United States) calibrated to 450 nm. Each sample was analyzed in triplicate, and results were classified as positive if the OD450 index met or exceeded the established cutoff value in the ELISA assay.

2.7. Statistical analysis

The statistical evaluation of the outcome was conducted utilizing SPSS software, version 26.0. Chi-square tests were employed to explore the associations between infection and the variables under investigation. Variables that demonstrated a significant correlation with the frequency of toxocariasis were further examined as possible risk factors using univariate logistic regression analysis. Variables that yielded a P of 0.20 or less were subsequently incorporated as predictors in the final multivariate logistic regression model. A $P<0.05$ was considered to indicate statistical significance for the associations analyzed.

3. Results

3.1. Seroprevalence of toxocariasis

Out of a total of 300 children with eosinophilia, 35(11.7%) were found seropositive for toxocariasis infection, while 265(88.3%) did not have any infection. Among the children with toxocariasis, 4(11.4%) were under 4 years old, 23(65.7%) were between 4 and 8 years old, and 8(22.9%) were over 8 years old. In contrast, among those without toxocariasis infection, 34(12.8%) were under 4 years old, 172(64.9%) were between 4 and 8 years old, and 59(22.3%) were over 8 years old. The age distribution between the two groups did not demonstrate statistical significance (P=0.972), suggesting that there is no significant association between age and the incidence of toxocariasis (Table 1).

Out of the total number of individuals with toxocariasis infection, 25(71.4%) were boys and 10(28.6%) were girls. In the group without infection, 165(62.3%) were boys and 100(37.7%) were girls. The disparity in gender distribution between the two groups did not reach statistical significance (P=0.193), suggesting that there is no significant association between gender and the incidence of toxocariasis infection ($P>0.05$).

In children seropositive for toxocariasis, 27(77.1%) resided in urban areas, while 8(22.9%) lived in rural regions. Conversely, among children who were seronegative for toxocariasis, 177(66.8%) lived in urban areas, and 88(32.2%) resided in rural regions. The lack of correspondence in residential distribution between the two groups did not reach statistical significance (P=0.148), suggesting that there is no meaningful relationship between residence and the incidence of toxocariasis infection ($P>0.05$).

Concerning the relationship between consumption of unwashed fruits/vegetables and toxocariasis, it was found that among children with eosinophilia who were seropositive for toxocariasis, 7 participants (20%) reported not consuming unwashed fruits/vegetables, while 28 participants (80%) had a history of consuming unwashed fruits/vegetables. Conversely, among children without toxocariasis, 234 participants (88.3%) did not report consuming unwashed fruits/vegetables, whereas 31 participants (11.7%) indicated a history of consuming unwashed fruits/vegetables. The observed difference in unwashed fruits/vegetables consumption between the two groups was statistically significant ($P<0.01$), suggesting a significant association between unwashed fruits/vegetables consumption and the incidence of toxocariasis infection.

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Table 1. Frequency of anti-IgG-toxocara antibodies in children with eosinophilia according to demographic characteristics and related risk factors

Variables		No. (%)		P*
		Anti-Toxocara IgG Antibody		
		Positive	Negative	
Age (y)	>4	4(11.4)	34(12.8)	0.972
	4-8	23(65.7)	172(69.4)	
	>8	8(22.9)	59(22.3)	
Gender	Boy	25(71.4)	165(62.3)	0.193
	Girl	10(28.6)	100(37.7)	
Residence	Urban	27(77.1)	177(66.8)	0.148
	Rural	8(22.9)	88(33.2)	
Consumption of unwashed fruits/ vegetables	No	7(20)	237(88.3)	0.001*
	Yes	28(80)	31(11.7)	
Animal contact	No	30(85.7)	222(83.8)	0.498
	Yes	5(14.3)	43(16.2)	

*P<0.05 indicates a statistically significant difference.

Regarding animal contact, among children with eosinophilia who tested positive for toxocariasis, 30 participants (85.7%) reported no contact with animals, while 5 participants (14.3%) indicated that they had such contact. In the group without toxocariasis, 222 participants (83.8%) did not report any animal contact, whereas 43 participants (16.2%) did have contact with animals. The disparity in the frequency of animal contact observed between the two groups did not reach statistical significance (P=0.498).

3.2. Risk factors

In the univariate regression model, the odds ratio calculated to assess the relationship between age (P=0.975), gender (P=0.293), residence (P=0.221), and animal contact (P=0.769) with seropositivity to toxocariasis was not statistically significant. The univariate regression analysis also revealed a significant (P<0.001) odds ratio for the relationship between unwashed fruits/vegetables consumption and seropositivity to toxocariasis (Table 2). In order to assess the influence of independent variables while accounting for possible confounding factors, a multivariable logistic regression model was utilized (Table 2). This analysis revealed that gender, place of residence, and unwashed fruits/vegetables consumption were significant predictors of toxocariasis infection (P<0.05). Specifically, female participants exhibited a lower likelihood of contracting toxocariasis compared to male children [P=0.002, OR=0.184 (0.052-0543)]. Additionally, individuals residing in urban areas were determined to have a 4.54-fold increased likelihood of being infected with toxocariasis compared to those living in rural areas [P=0.010, OR=4.546 (1.433-9.419)]. Furthermore, individuals who did not consume unwashed fruits/vegetables demonstrated a reduced risk of toxocariasis infection [P=0.001, OR=0.016 (0.005-0.048)].

4. Discussion

Today, it has been proven that eosinophilia is one of the primary clinical manifestations of toxocariasis, particularly in pediatric populations [4]. Current evidence indicates that *Toxocara* larvae induce significant eosinophilia through their influence on IgE antibodies and IL-5 cytokines, which may contribute to allergic complications [5, 6]. Children in the early years of life, particularly during the first decade, demonstrate heightened vulnerability to toxocariasis [10]. This increased susceptibility can be attributed to their propensity for geophagy, regular interactions with animals, and participation in activities that involve soil [10]. Given the notable preva-

Table 2. Multivariable logistic regression analysis for risk factors associated with toxocariasis seropositivity in children with eosinophilia from Western Iran

Variables	Univariable Analysis		Multivariable Analysis	
	Odds Ratio (95% CI)	P	Odds Ratio (95% CI)	P
Age	0.986 (0.419, 2.324)	0.975	1.141 (0.350, 3.722)	0.827
Gender	0.660 (0.304, 1.432)	0.293	0.184 (0.062, 0.543)	0.002*
Residence	1.678 (0.732, 3.846)	0.221	4.546 (1.433, 9.419)	0.010*
Consumption of unwashed fruits/vegetables	0.033 (0.013, 0.082)	0.000*	0.016 (0.005, 0.048)	0.000*
Animal contact	1.162 (0.427, 3.164)	0.769	1.074 (0.312, 3.694)	0.910

*P<0.05 indicates a statistically significant difference.

lence of the *Toxocara* parasite in this region, this study aimed to investigate the seroepidemiology of toxocariasis and its risk factors in children with hypereosinophilia in Lorestan Province, Western Iran.

Our serological tests showed that out of 300 children with eosinophilia, 35(11.7%) were found seropositive for toxocariasis infection, while 265(88.3%) did not have any infection. Prior seroepidemiological investigations conducted in Iran indicate that the prevalence of this infection among humans ranges from 2% to 25% across various regions and provinces. For example, Sarkari et al. (2015) showed that anti-*Toxocara* antibodies were found in two (2%) and three (3%) of the hypereosinophilic patients (eosinophil count >10%) and normal individuals (eosinophil count 1-6%), respectively. The findings demonstrated no substantial correlation between hypereosinophilia and the presence of anti-*Toxocara* antibodies [12]. In the study by Mesibi et al. (2011), the prevalence of *Toxocara migrans* larvae in 100 individuals with hypereosinophilia in Arak City, Markazi Province, Iran was 16%, compared to 0% in those with normal eosinophil levels [13]. In a study conducted in Shiraz involving children aged 6 to 13 years, the prevalence of toxocariasis was reported to be 25.6%. This prevalence was further broken down into 20.2% in rural areas and 30.1% in urban areas [14]. Akhlaghi et al. (2015) reported that among children aged 2 to 12 years in the Mahidasht region of Kermanshah, the prevalence of toxocariasis infection was 8.6% [15]. In another study conducted by Ghafari Naqneh et al. (2014), the prevalence of *T. canis* infection in children aged 2 to 14 years in Chaharmahal and Bakhtiari Province was reported to be 11 cases (2%) [16]. In a study conducted by Alavi et al. (2009), the serum prevalence of *T. canis* among primary school children aged 6 to 15 years in both urban and rural areas of Ahvaz City was reported to be 2% [17]. Regarding the incidence of toxocariasis in Lorestan Province, Iran, Mahmoudvand et al (2016) reported that among 316 children aged 2 to 15 years in the region, 14 children, representing 4.4% of the sample, were seropositive for toxocariasis [11]. In the research conducted by Abbasi et al. (2016), it was found that the prevalence of anti-*Toxocara* antibodies among 200 children under the age of 15 exhibiting skin and respiratory allergic symptoms in Zanjan Province, Iran, was 4.5%. This prevalence was significantly greater than that observed in healthy children (0.8%) [18]. The discrepancy in the reported prevalence rates between prior research and the present study may be attributed to several factors, including sociocultural behaviors, the specific target population examined, the geographical locations of the studies, the climatic conditions prevalent in the study areas, and the methodologies employed to assess the prevalence of toxocariasis [9].

Our results showed that gender, place of residence, and unwashed fruits/vegetables consumption were significant risk factors for toxocariasis (P<0.05). In the study conducted by Mahmoudvand et al. (2016) in the same area under study, the results showed that settling in rural areas and contact with dogs were the main risk factors correlated with *T. canis* seropositivity [17]. Sarkari et al. (2015) found no significant correlation between seropositivity to *Toxocara* and the age or sex of patients exhibiting hypereosinophilia, as well as among a control group of 100 healthy individuals [11]. Song et al. (2020) further established that the consumption of raw meat or animal livers, along with heavy alcohol consumption, was identified as risk factors for toxocariasis in individuals exhibiting eosinophilia [19]. It has been demonstrated that boys exhibit a greater susceptibility to toxocariasis compared to girls, which can be attributed to their increased levels of physical activity, particularly

in outdoor environments, leading to heightened exposure to soil [10, 20]. Conversely, it is important to note that interaction with the primary hosts of this infection, namely cats and dogs, as well as the consumption of raw fruits and vegetables contaminated with *Toxocara* eggs shed in the feces of these definitive hosts, represents the principal modes of transmission identified in our study [10, 21]. Furthermore, these two factors have been demonstrated to be the most significant risk factors for toxocariasis in individuals exhibiting eosinophilia. The main limitations of the current study are the smaller sample size and the need for additional clinical diagnostic tests to confirm the disease. It should be mentioned that children who tested positive for the presence of anti-*Toxocara* IgG antibody were referred to a specialist for follow-up and, if necessary, treatment.

5. Conclusion

The findings of our study indicated a significant seroprevalence of toxocariasis among children exhibiting eosinophilia in western Iran. This suggests that toxocariasis should be regarded as a potential etiological factor for eosinophilia, particularly in patients with a history of consuming unwashed fruits/vegetables and those living in rural areas in Iran.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Lorestan University of Medical Sciences, Khorramabad, Iran (Code: IR.LUMS.REC.1398.213). Furthermore, written informed consent was secured from the parents of the participating children prior to the collection of blood samples.

Data availability

The dataset presented in the study is available upon request from the corresponding author during submission or after publication.

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Authors' contributions

Supervision and methodology: Hossein Mahmoudvand and Yosra Raziani; Experiments and data collection: Azadeh Sepahvand and Parastoo Baharvand; Writing: Hossein Mahmoudvand; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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