



# Evaluation of Visceral Toxocariasis Seropositivity and Possible Risk Factors in Patients with a Clinical Preliminary Diagnosis of Cystic Echinococcosis\*

*Kistik Ekinokokkoz Klinik Ön Tanılı Hastalarda Visceral Toxocariasis Seropozitifliği ve Olası Risk Faktörlerinin Değerlendirilmesi\**

Yasemin Büyük<sup>1</sup>, Mukremin Ozkan Arslan<sup>2</sup>

<sup>1</sup>Department of Medical Parasitology, Kafkas University Health Sciences Institute; <sup>2</sup>Department of Parasitology, Kafkas University Faculty of Medicine, Kars, Türkiye

## ABSTRACT

**Aim:** This study aimed to evaluate the seropositivity and possible risk factors for visceral toxocariasis in patients with a clinical preliminary diagnosis of cystic echinococcosis.

**Material and Methods:** Between October 2022 and December 2023, 184 blood serum samples, which were taken from patients with a clinical preliminary diagnosis of cystic echinococcosis and sent to the Medical Parasitology Laboratory of the Health Research and Training Hospital of Kafkas University, were analyzed for anti-Toxocara canis IgG using ELISA. Anti-Ascaris lumbricoides IgG ELISA was applied to the positive samples to differentiate cross-reactions.

**Results:** In this study, anti-T.canis IgG positivity was detected in 73 (39.67%) of the 184 serum samples. Among the sera determined to be positive for anti-T.canis IgG, mixed infection with A.lumbricoides was detected in 38 (52.05%) samples, and cross-reactions were identified in 10 (13.7%) samples. The study revealed the overall positivity rate for anti-T.canis IgG alone as 23.97% (35/146), with rates of 24.47% (23/94) in women and 23.08% (12/52) in men. Out of the 6 samples positive for cystic echinococcosis (6/184; 3.26%), 3 (3/6; 50%) were also seropositive for visceral toxocariasis. Anti-T. canis IgG was detected at higher rates in women, individuals over 65 years of age, illiterates, farmers, and rural inhabitants (P >0.05). The levels of anti-T.canis IgG were significantly higher in individuals with detached houses with gardens, those who kept dogs in their gardens, those with vegetable patches, those with neighboring gardens and surroundings with dogs, and those engaged in soil-related garden work (P <0.05).

**Conclusions:** In Kars and its surroundings, visceral toxocariasis was significant for public health, especially among individuals in contact with stray dogs and in soil-related occupations. Further studies are needed to determine the correlation between visceral toxocariasis and cystic echinococcosis, which share similar epidemiologic risks and clinical complaints.

**Key words:** anti-Toxocara canis IgG; risk factor; visceral toxocariasis; cystic echinococcosis

## ÖZET

**Amaç:** Bu çalışmada kistik ekinokokkoz klinik ön tanılı hastalarda visceral toxocariasis seropozitifliği ve olası risk faktörlerinin değerlendirilmesi amaçlandı.

**Gereç ve Yöntem:** Kafkas Üniversitesi Sağlık Araştırma ve Uygulama Hastanesi Tıbbi Parazitoloji laboratuvarına Ekim 2022-Aralık 2023 tarihlerinde kistik ekinokokkoz klinik ön tanılı hastalardan gönderilen 184 kan serum örneği anti-Toxocara canis IgG yönünden ELISA ile analiz edildi. Pozitif örneklerle çapraz reaksiyonları ayırt etmek için anti-Ascaris lumbricoides IgG ELISA uygulandı.

**Bulgular:** Çalışmada, 184 serum örneğinin 73 (%39,67)'ünde anti-T.canis IgG pozitifliği saptandı. Anti-T.canis IgG saptanan örneklerden 38 (%52,05)'inde A.lumbricoides ile miks enfeksiyon, 10 (%13,7)'ünde ise çapraz reaksiyon belirlendi. Araştırmada sadece anti-T.canis IgG pozitifliği %23,97 (35/146) bulunmuş olup, bu oran kadınlarda %24,47 (23/94) ve erkeklerde %23,08 (12/52) olarak belirlendi. Kistik ekinokokkoz pozitif bulunan altı (6/184; %3,26) örneğin üçünde (3/6; %50) visceral toxocariasis seropozitif saptandı. Anti-T.canis IgG; kadınlarda, 65 yaş üstü bireylerde, okur-yazar olmayanlarda, çiftçilerde ve kırsalda yaşayanlarda daha yüksek oranda tespit edildi (P >0,05). Müstakil bahçeli evi olanlarda, evinin bahçesinde köpek bulunanlarda, evinin bahçesinde bostan varlığı olanlarda, komşu bahçesi ve çevresinde köpek varlığı olanlarda ve toprakla irtibatlı bahçe işleri ile uğraşı olanlarda daha yüksek oranda bulunan anti-T.canis IgG pozitifliği istatistiksel olarak anlamlı bulundu (P <0,05).

**Sonuç:** Kars ve çevresinde visceral toxocariasisin özellikle sahihsiz köpek irtibatlı ve toprak temaslı işlerle uğraşanlarda halk sağlığı yönünden önemli olduğu belirlendi. Benzer epidemiyolojik riskler ve klinik şikâyetleri paylaşan visceral toxocariasis ve kistik ekinokokkoz arasındaki korelasyonunun saptanması için daha fazla çalışmaya ihtiyaç duyulmaktadır.

**Anahtar kelimeler:** anti-Toxocara canis IgG; risk faktör; visceral toxocariasis; kistik ekinokokkoz

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**İletişim/Contact:** Yasemin Büyük, Kafkas University Health Sciences Institute Department of Medical Parasitology, Kars, 36000, Türkiye. • **Tel:** 0474 242 68 12 • **E-mail:** yaseminsabin46@hotmail.com • **Geliş/Received:** 15.01.2025 • **Kabul/Accepted:** 11.03.2025

**ORCID:** Yasemin Büyük: 0000-0002-7986-9686 • Mukremin Özkan Arslan: 0000-0002-6447-5561

## Introduction

Human toxocariasis, also known as visceral toxocariasis, is a zoonotic parasitic infection of global public health significance and is caused by the larvae of the roundworms *Toxocara canis* and *Toxocara cati*. Dogs serve as the primary source of infection for humans. Humans are described among the paratenic hosts of these ascarids. Toxocariasis is transmitted to humans via the accidental ingestion of embryonated eggs, which are shed in the feces of infected dogs and gain infectivity by larval development when nested in the soil. The encapsulated larvae hatch from the eggs in the small intestine and, after perforating the intestinal wall and entering the vascular system, are disseminated throughout the body in the bloodstream. Visceral larva migrans (VLM) is an important clinical form of infection caused by damage caused by migrating larvae to several visceral organs<sup>1</sup>.

A global assessment demonstrated that Indonesia, Malaysia, and Papua New Guinea ranked first in *Toxocara* spp. prevalence with seropositivity rates higher than 60%. Nigeria, Gabon, Colombia, Romania and Vietnam followed next, with prevalence rates ranging from 41% to 60%. *Toxocara* seropositivity in Türkiye is similar to that in Russia, Australia and Canada, ranging between 6–15%<sup>2</sup>. Several studies have been conducted on the seroprevalence of human toxocariasis in Türkiye<sup>3–6</sup>, and seropositivity has been reported as 32.3–48.4% in the Istanbul province<sup>7</sup> and 45.9% in the Elazığ province<sup>8</sup>.

In the Kars region, the prevalence of adult *T.canis* parasites has been detected as 50% in dogs<sup>9</sup> and 20% in foxes<sup>10</sup>, yet no regional data is available on human toxocariasis. On the other hand, the prevalence of cystic echinococcosis (CE) in patients with a clinical preliminary diagnosis of CE has been determined to be 14.9% in the Kars region<sup>11</sup>. Given this finding, the design of the present study was based on the assumption that visceral toxocariasis and CE, two major parasitic zoonoses of canine origin, are associated with each other.

The present study was aimed at the serological investigation (by the enzyme-linked immunosorbent assay, ELISA) of visceral toxocariasis in patients who were admitted to the polyclinics of the Health Research and Training Hospital of Kafkas University located in the city center of Kars, with various complaints and were either preliminarily diagnosed with or suspected of having CE, as well as the determination of seropositivity for anti-*Toxocara canis* IgG, and the assessment of the risk factors involved in the epidemiology of human toxocariasis.

## Material and Methods

### Ethics statement

The study received ethical approval from the Kafkas University Non-Interventional Clinical Research Ethics Committee, with decision number “80576354-050-99/107.”

### Study material

This study was conducted at the Health Research and Training Hospital of Kafkas University, located in the city center of Kars. The investigation was designed as a prospective study on outpatients admitted to the hospital's various polyclinics with a clinical preliminary diagnosis or suspicion of CE and for whom a laboratory diagnosis of CE was requested from the Parasitology Laboratory.

The study material comprised the venous blood samples of 184 patients aged 9–90 years and serologically diagnosed with CE after being referred to the Medical Parasitology Laboratory between October 2022 and December 2023. The blood samples underwent centrifugation at 3000 rpm/min at the Medical Parasitology Laboratory of the Health Research and Training Hospital of Kafkas University for the extraction of sera. The extracted serum samples were transferred in 200–500 ml volumes into sterile microcentrifuge tubes, labelled, and stored at -20°C until being analyzed.

### ELISA analysis

The blood serum samples were analyzed to determine the presence of anti-*T.canis* IgG was performed using the NovaLisa *Toxocara canis* IgG ELISA Kit (Novtcg0450, NovaTec Immundiagnostica GmbH, Germany) according to the manufacturer's instructions. The blood sera, which were determined to contain anti-*T.canis* IgG were further analyzed for the presence of anti-*A.lumbricoides* IgG antibodies using the NovaLisa *A.lumbricoides* IgG ELISA kit (Novascg0020, NovaTec Immundiagnostica GmbH, Germany) to differentiate possible cross-reactions arising from *A.lumbricoides* infection.

### IHA analysis

The routine laboratory diagnosis of CE was also performed at the Medical Parasitology Laboratory of the Health Research and Training Hospital of Kafkas University by means of the indirect hemagglutination assay (IHA) (Siemens Cellognost Echinococ, antibody

titers of  $\geq 1/128$  were considered positive, and titers of  $1/64$  were considered suspect). The results were used for the comparative analysis with anti-*T.canis* IgG seropositivity.

### Statistical Analysis

The statistical analysis of the study data was performed with the chi-square test of independence. The direction of correlation between the categorical variables,  $P < 0.05$ , the strength of correlation (fi coefficient) and the odds ratio was determined. The study design was based on analyzing all blood serum samples of patients with a preliminary diagnosis of CE for anti-*T.canis* IgG antibodies within the set time period, no sample size was predetermined.

The study was conducted pursuant to the institutional permit granted by the hospital, the approval of the Ethics Board of Kafkas University, and the signed voluntary participation forms of the patients. A questionnaire was developed and administered to evaluate demographic data, socioeconomic characteristics and visceral toxocarasis risk factors. Patients, either for whom data records were not available in the hospital registration system or who refused to share information, were excluded from the study, such that the analyses were based on the number of answers (n) received for each question.

## Results

In this study, 73 (39.67%) samples were determined to be positive for anti-*T.canis* IgG and no statistically significant correlation was determined between seropositivity and sex ( $P > 0.05$ ) (Table 1).

Out of the 73 samples which were determined to be positive for anti-*T.canis* IgG, 38 (52.05%) displayed mixed infection with *A.lumbricoides*, and 10 (13.7%) presented with cross-reactions between *A.lumbricoides*. In this study, the overall rate of seropositivity for anti-*T.canis* IgG alone was determined to be 23.97% (35/146) (Table 2).

In this study, anti-*T.canis* IgG seropositivity was found to be 4.3% (27/111) in the Kars province and 22.2% (2/9) in the Ardahan province. In the study, the “n” value varied with the answers given to the questionnaire items. The distribution of the anti-*T.canis* IgG-positive samples for the demographic characteristics of the patients is presented in Table 3.

It was ascertained that the risk of acquiring visceral toxocarasis was 0.28-fold higher in individuals living in detached houses with a garden, when compared to those living in an apartment/flat, 2.54-fold higher in people keeping dogs in the garden of their houses, when compared to those with no dog in the house garden, 2.41-fold higher in individuals known to be engaged in growing greens and have a vegetable patch as part of their house garden, when compared to those with no such vegetable patch and cultivation activity, 3.07-fold higher in individuals with dogs present in the neighboring garden(s) and surroundings, compared to those not exposed to such contact, and 2.9-fold higher in people engaged in soil-related garden work, compared to those with no such activity (Table 3).

The present study demonstrated that most patients were determined to be seropositive for anti-*T.canis* IgG suffers from associating hepatic and pulmonary

**Table 1.** Anti-*T. canis* IgG ELISA results of the blood serum samples positive for CE

Sex	Number of samples (%)	Positive (%)	Suspect (%)	Negative (%)	P- value
Female	117 (63.59)	45 (38.46)	6 (5.13)	66 (56.41)	> 0.05
Male	67 (36.41)	28 (41.79)	4 (5.97)	35 (52.24)	
Overall	184	73 (39.67)	10 (5.43)	101 (54.89)	

**Table 2.** Exclusively seropositivity for anti-*T.canis* IgG after exclusion of co-infection with *A.lumbricoides*

Sex	Anti- <i>T.canis</i> IgG			
	Number of samples (%)	Positive (%)	Suspect (%)	Negative (%)
Female	94 (64.38)	23 (24.47)	6 (6.38)	65 (69.14)
Male	52 (35.62)	12 (23.08)	4 (7.69)	36 (69.23)
Overall	146	35 (23.97)	10 (6.85)	101 (69.18)

**Table 3.** Seropositivity of *T.canis* according to sociodemographic characteristics and possible risk factors

Characteristic	Anti-T.canis IgG positivity		P value / Odds ratio
	n	%	
<b>Sex</b> (n: 146)			
Female (n= 94)	23	24.47	> 0.05
Male (n= 52)	12	23.08	
<b>Age</b> (n: 120)			
9-24 years (n= 7)	0	0	> 0.05
25-44 years (n= 42)	7	16.67	
45-64 years (n= 45)	12	26.67	
65 years and older (n= 26)	10	38.46	
<b>Educational level</b> (n: 120)			
Illiterate (n= 35)	12	34.29	> 0.05
Primary school - Secondary school (n= 55)	12	21.82	
High school (n= 19)	4	21.05	
University (n= 11)	1	9.09	
<b>Occupation</b> (n: 120)			
Housewife (n= 73)	20	27.4	> 0.05
Farmer (n= 21)	6	28.57	
Civil servant (n= 12)	2	16.67	
Student (n= 5)	0	0	
Other (n= 9)	1	11.11	
<b>Residency</b> (n: 120)			
Urban (district/city center) (n=63)	13	20.63	> 0.05
Rural (Village) (n= 57)	16	28.07	
<b>Dwelling type</b> (n: 120)			
Apartment/flat (n= 37)	4	10.81	< 0.05 / 0.28
Detached house with garden (n= 83)	25	30.12	
<b>Presence of dog(s) as house pet(s)</b> (n: 120)			
Yes (n= 0)	0	0	N/A
No (n= 120)	29	24.17	
<b>Presence of dog(s) in house garden</b> (n: 120)			
Yes (n= 42)	15	35.71	< 0.05 / 2.54
No (n= 78)	14	17.95	
<b>Presence of vegetable patch as part of house garden (engagement in the growing of greens)</b> (n: 120)			
Yes (n= 43)	15	34.88	< 0.05 / 2.41
No (n= 77)	14	18.18	
<b>Presence of dog(s) in neighboring gardens and surroundings</b> (n: 120)			
Yes (n= 86)	25	29.07	< 0.05 / 3.07
No (n= 34)	4	11.76	
<b>Engagement in soil-related garden work</b> (n: 120)			
Yes (n= 55)	19	34.55	< 0.05 / 2.9
No (n= 65)	10	15.38	

diseases, rheumatoid disease, gastroenteritis and allergic skin disorders. It was ascertained that 20 (22.99%) of the 87 patients were referred to the General Surgery Polyclinic, and 11 (22.45%) of the 49 patients were referred to the Internal Medicine Polyclinic. Overall, a large majority (88.6%, 31/35) of the 35 samples with visceral toxocariasis had been referred to by the General Surgery and Internal Medicine Polyclinics.

In this study, out of the 6 samples (6/184; 3.26%) determined to be seropositive for CE, 3 (3/6; 50%) were also ascertained to be seropositive for visceral toxocariasis.

## Discussion

Human visceral toxocariasis is known to be more prevalent in tropical and subtropical regions, areas with limited control of the dog population, and low- and middle-income countries<sup>1</sup>. While the global prevalence of anti-*Toxocara* serum antibodies has been reported as 19%, regional prevalence has been detected to be highest in Africa (37.7%), lowest in the Eastern Mediterranean (8.2%), and to occur at levels of 34.1%, 24.2%, 22.8% and 10.5% in Southeastern Asia, the Western Pacific, America and Europe, respectively, with Nigeria, Romania, Argentina and Egypt displaying the highest country seroprevalences of 44%, 42%, 35% and 32%, respectively, and Japan, Spain and Italy the lowest detected seroprevalence of 4%<sup>12</sup>. The same researchers reported the prevalence in Türkiye as 7%. In a meta-analysis conducted by Ulloque-Badaracco et al.<sup>13</sup> for a period from 1990 to 2022, the general seroprevalence of human toxocariasis in Latin America and the Caribbean was determined as 31%, and seroprevalence was reported to be higher in dog owners.

Said et al.<sup>14</sup> reported the overall seroprevalence of anti-*T.canis* antibodies in different occupational groups in northwest Pakistan as 14.2% and demonstrated significant differences between the seropositivity rates of several subgroups about several variables such as income distribution, educational background and involvement in agricultural labor. Pezeshkian et al.<sup>15</sup> reported a toxocariasis seroprevalence of 5.8% in the adult population of the Kavar region of southern Iran. Luca et al.<sup>16</sup> reported a seropositivity rate of 22.64% in Romania and indicated that the elderly were affected more than children.

The seroprevalence of visceral toxocariasis has been reported as 5.14% in primary school children from China,<sup>17</sup> 7% in eosinophilic children from northwest

Iran,<sup>18</sup> and 45.9% in the homeless and 27.8% in animal shelter workers from Brazil<sup>19</sup>.

Previous researches from Türkiye has investigated the correlation of toxocariasis prevalence with several potential risk factors, including sex, age, socioeconomic status, occupation, pet animal ownership and geophagy<sup>20</sup>. To the authors' knowledge, there is no previous study on human *Toxocara* infections in the Kars region of Türkiye. On the other hand, seropositivity was reported as 48.4% in hypereosinophilic individuals from Istanbul in 2005<sup>7</sup> and 45.9% in schizophrenic patients from Elazığ in 2008<sup>8</sup>. Anti-*T.canis* IgG seropositivity rates were reported as 12% in Bolu<sup>6</sup>, 15% in Van<sup>4</sup>, 16% in Isparta<sup>21</sup>, 8% in farm workers from Muğla<sup>22</sup>, 17.8% in chronic urticaria patients from Istanbul<sup>23</sup>, 9.7% in asthma patients from Ankara<sup>24</sup> and 21.4% in different age groups from Kayseri<sup>25</sup>. Studies on the prevalence of visceral toxocariasis in children from Türkiye have reported rates of 7.6% in Kütahya<sup>3</sup>, 12.95% in Izmir<sup>26</sup> and 32.3% in Sivas<sup>27</sup>.

All of the patients enrolled in the present study were preliminarily diagnosed with CE and suffered from various clinical conditions and signs, including, among others, liver and lung diseases, gastrointestinal disorders and skin infections. The present study demonstrated a seropositivity rate of 23.97%, which is close to the global seroprevalence (19%) reported by Rostami et al.<sup>12</sup>, and also falls within the seroprevalence range reported for European countries (4%–42%). However, no study has investigated the prevalence of *T.canis* in individuals with a prediagnosis of CE, and no head-to-head comparison has been made. On the other hand, the fact that our study was conducted in individuals with a prediagnosis of CE who had common epidemiologic risk factors and similar clinical symptoms made the group studied a special/selected group. Therefore, a higher seropositivity rate is expected to be detected than in other studies.

Despite having a high diagnostic accuracy (98.63% specificity and 96.92% sensitivity), the ELISA kit used in the present study was indicated by the manufacturer to produce cross-reactions for antibodies against *A.lumbricoides* and *Schistosoma*. In view of this possibility, the seropositive samples were subjected to a second analysis by ELISA, such that the serum samples were determined to be positive for anti-*T.canis* IgG was analyzed a second time by ELISA for anti-*A.lumbricoides* IgG antibodies. Due to *Schistosoma* infections not occurring in Türkiye, the anti-*T.canis* IgG-positive serum

samples were not analyzed for *Schistosoma* spp. As a result, 10 (10/73; 13.7%) of the anti-*T.canis* IgG-positive serum samples were suspected of being positive for anti-*A.lumbricoides* IgG antibodies, and these samples were assessed as having produced cross-reactions. On the other hand, we determined mixed infection with *T.canis* and *A.lumbricoides* in 38 (38/73, 52.05%) serum samples. This finding agrees with literature reports indicating individuals infected with *Toxocara* spp can be concurrently infected with the parasites *Toxoplasma gondii* and *Fasciola hepatica*<sup>28,29</sup>.

As is the case with several other parasitic diseases, the epidemiology of visceral toxocariasis is affected by multiple risk factors. Although it is generally reported that visceral toxocariasis seropositivity is higher in women compared to men<sup>12,30,31</sup>, the exact opposite has also been reported<sup>15,19,32</sup>. The present study did not show any statistically significant difference between men and women in the prevalence of visceral toxocariasis ( $P > 0.05$ ). While the occurrence of visceral toxocariasis has been reported to be high among children<sup>25,33,34</sup>, due to increased exposure to parasite eggs with age, senile individuals have been reported to show a higher rate of seropositivity<sup>14,16,19,35</sup>. In agreement with these reports, in the present study, while anti-*T.canis* IgG positivity was not detected in the individuals aged 9–24 years, positivity was 16.67% in those aged 25–44 years, 26.67% in those aged 45–64 years, and 38.46% in those aged 65 years and older.

Higher visceral toxocariasis seropositivity rates have been reported in hunters, farmers and animal shelter workers<sup>33</sup>, housewives<sup>15</sup>, and rural inhabitants<sup>17,36,37</sup>. In agreement with these reports, the present study demonstrated an anti-*T.canis* IgG positivity rate of 28.07% in rural inhabitants and 20.63% in urban inhabitants ( $P > 0.05$ ).

Visceral toxocariasis seropositivity has been reported to be higher in dwellings of low quality and with poor hygiene conditions<sup>38</sup>. In the present study, anti-*T.canis* IgG positivity was determined to be 10.81% in those living in a flat and 30.12% in those living in a detached house with a garden ( $P < 0.05$ , odds ratio, 0.28).

Multiple studies carried out in Türkiye and different regions of the world have pointed out significantly higher *Toxocara* positivity rates in individuals who either own dogs as pets, feed/take care of dogs inside or outside their houses, have close contact with dogs or share their living space with dogs<sup>12,14,17,31,33,34</sup>. In the

present study, a statistically significant correlation was determined between anti-*T.canis* IgG positivity and the presence of dogs in the house garden, neighbouring garden or surroundings of the patients ( $P < 0.05$ ). The prevalence of visceral toxocariasis was 2.54-fold (odds ratio, 2.54) higher in patients keeping dogs in the garden of their house compared to those with no dog in their garden, and 3.07-fold higher (odds ratio, 3.07) in those with dogs in neighbouring gardens compared to those with no dog in neighbouring gardens. These findings show that the presence of dogs in the garden of the house of the patients or their neighbor's house significantly affected visceral toxocariasis seropositivity, and in agreement with the previous studies referred to above, demonstrated that these variables were not independent of each other.

Eggs shed in infected dogs' feces become infective within 3–4 weeks at a temperature range of 15–35°C. For this reason, soil-related garden work has been reported to increase visceral toxocariasis seropositivity<sup>33,38,39</sup>. Hence, in the present study, a higher rate of visceral toxocariasis was determined in patients involved in soil-related garden work and those with a vegetable patch as part of their house garden ( $P < 0.05$ ).

Seropositivity rates for visceral toxocariasis and CE, which usually share a common patient history of contact with dogs, similar epidemiological risks and common clinical complaints such as liver and lung diseases, were determined not to show any statistically significant correlation with each other in the present study. Nevertheless, 3 (50%) out of the 6 patients preliminarily diagnosed with CE had been determined to carry anti-*T.canis* IgG antibodies were considered to be an important finding. However, the low CE positivity rate (3.26%) made comparing and interpreting data difficult. Thus, there is a need for further studies on this particular issue.

## Conclusion

A significantly high rate of visceral toxocariasis seropositivity was determined in the residents of Kars and its vicinity, and it was ascertained that several demographic and socioeconomic factors were involved in the epidemiology of this parasitic infection. It was concluded that the investigation of the correlation of visceral toxocariasis with CE should be based on a larger sample size and that further research is required from the one health perspective.

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### Authors' Contribution

The authors share the responsibility for the manuscript.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Conflict of Interests

The authors declare no potential conflicts of interest regarding this article.

### Disclaimer

The content is solely the responsibility of the authors.

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