

Prevalence of toxocariasis and its related risk factors in humans, dogs and cats in northeastern Iran: a population-based study

Mohammad Reza Rezaeiemanesh^a, Monavar Afzalaghaee^b, Sara Hamidi^c, Ameneh Eshaghzadeh^d, Maryam Paydar^{b,e} and Seyed Hossein Hejazi^{a,f,*}

^aDepartment of Parasitology and Mycology, School of Medicine, Isfahan University of Medical Science, Isfahan, Iran; ^bSocial Determinant of Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; ^cDepartment of Laboratory Sciences, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran; ^dHealth Network Laboratory of Roshtkhar, Mashhad University of Medical Sciences, Mashhad, Iran; ^eDepartment of Environmental Health Engineering, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran; ^fSkin Diseases and Leishmaniasis Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

*Corresponding author: Tel.: +983137929173; Mobile phone: +989133118711; E-mail: hejazi@med.mui.ac.ir

Received 3 October 2018; revised 4 January 2019; editorial decision 13 February 2019; accepted 20 February 2019

Background: Toxocariasis is one of the most common zoonotic infections in dogs and cats. The objectives of this study were to determine the prevalence of anti-*Toxocara* antibodies and identify the epidemiological and clinical features and related risk factors of toxocariasis in the general population along with the prevalence of this infection in dogs and cats in northeast Iran.

Methods: In this cross-sectional study, anti-*Toxocara* antibodies from 1150 urban and rural participants were examined using a commercial enzyme-linked immunosorbent assay. In addition, the stool specimens of 538 dogs and 236 cats were tested using the formalin–ether concentration technique. Sociodemographic data and related risk factors were collected using a structured questionnaire and the data on clinical manifestations were obtained from personal health records.

Results: Anti-*Toxocara* antibodies were detected in 7.2% (83/1150) of the study population. Risk factors for seropositivity based on multivariable analysis were younger age ($p=0.019$), living in rural areas ($p=0.048$), occupations with high exposure risk to toxocariasis ($p=0.001$), contact with dogs or cats ($p=0.003$), contact with soil ($p=0.001$), history of geophagy ($p=0.001$) and the consumption of raw or undercooked liver ($p=0.001$). The prevalence of symptoms such as asthma, shortness of breath, blurred vision, abdominal pain and epilepsy were significantly higher in seropositive individuals compared with seronegative ones. *Toxocara* eggs were found in 15.1% (81/538) of dogs and 34.7% (82/236) of cats. A significant relationship was found between infection in dogs and cats and age, type, place of residence and anthelmintic consumption.

Conclusions: Identification of anti-*Toxocara* antibodies in the general population and the presence of confirmed infections in dogs and cats indicates exposure to *Toxocara* infection as a public health problem in northeast Iran.

Keywords: cat, dog, Iran, risk factors, seroprevalence, toxocariasis

Introduction

Toxocariasis is one of the most common zoonotic parasitic infections around the world.¹ The causative agents of this infection, *Toxocara canis* and *Toxocara cati*, parasitize dogs and cats, respectively. The eggs leave the body with the faeces and enter the soil, where infective larvae develop within 2–5 weeks, depending on climatic conditions.^{2,3}

Transmission of infection to humans commonly occurs through the accidental ingestion of infectious eggs with soil, water or vegetables and by ingesting encapsulated infective larvae in raw or undercooked meat or organic meat from paratenic hosts such as chickens, sheep, cattle and rabbits.^{4–6}

In human infections, released larvae (from eggs or granuloma) penetrate the wall of the small intestine and migrate to different organs and tissues such as the liver, lungs, muscles, eyes and

central nervous system. The larvae do not undergo any further development in these sites but can cause severe local reactions. Four types of clinical syndromes, including visceral larvae migrans, ocular larvae migrans, neurotoxocariasis and covert toxocariasis, have been identified in patients with toxocarosis.⁷⁻⁹

Since the clinical symptoms are non-specific, coproscopic examination is not applicable due to the incomplete life cycle of the parasite in the human, and since tissue biopsy is an invasive method, the diagnosis of this infection in humans is usually based on clinical presentation, exposure history and laboratory tests. The enzyme-linked immunosorbent assay (ELISA) technique using *T. canis* larval excretory-secretory (TES) antigens is widely used in seroepidemiological studies.^{10,11}

The prevalence of human toxocariasis varies from 1.6–2.4% in developed countries such as Japan and Denmark to 81–87% in Nepal and the Marshall Islands.¹²⁻¹⁵ A prevalence rate of 1–45% in adult dogs, 3.2–91% in cats and 51–100% in puppies and kittens has been reported from some countries such as Nigeria, India and China.¹⁶⁻¹⁹ Most epidemiological studies on human toxocariasis in Iran have been carried out on children and the prevalence of anti-toxocarial antibodies ranges from 1.4% in children in Isfahan province²⁰ to 29.5% in children in East Azerbaijan province.²¹

Toxocariasis has been considered a neglected disease in many countries due to the limited attention paid to its surveillance, prevention and treatment. Although climatic, geographical and environmental conditions play an important role in its prevalence, contact with contaminated soil, water or vegetables; dog and cat ownership; age; personal habits; socio-economic status; education level and occupation are other major risk factors associated with this infection.^{8,22,23}

The prevalence of this infection in the general population of Iran is unknown and there is very little information available about toxocariasis in Khorasan Razavi province. Population-based studies are rare and most epidemiological studies of *Toxocara* in Iran have been performed in special groups such as preschool children, school children, asthmatic patients or patients with atopy and eosinophilia.

The aim of this study was to determine *Toxocara* seroprevalence and possible risk factors in the general population. A parallel investigation was also conducted aimed at detecting the rate of infected animals.

Materials and methods

Study area and population

Khorasan Razavi province, with an area of 118 854 km², is the fifth largest province of Iran and, with approximately 6 400 000 inhabitants, is the second most populous province in the country. This region generally has a cold, semi-arid climate. The average annual precipitation is 75–390 mm, the average relative humidity is 55% and the average temperature of the region is 15.9°C, ranging from –22.2°C to 43.1°C.

From October 2017 to July 2018, sampling of 10 counties including their subset cities and villages was done using the stratified cluster method. The province was initially divided into five regions (strata) according to climatic characteristics. Cluster sampling was performed in each region in proportion to its

population, including at least two counties (cluster) in each region and a minimum of 10 cities and surrounding villages (Figure 1). Individuals ≥ 2 y of age with no contraindication for blood sampling who were referring to health centres entered the study after giving written informed consent. All participants were native to the urban or rural area selected for sampling. Animals were enrolled in the study after obtaining the owner's consent; they had no age restrictions and were from every breed and type. In all cases, a relative precision of 0.1 and a confidence level of 90% was used. Based on a recent study²⁴ reporting a prevalence rate of 20% in humans, 18% in dogs and 29% in cats and applying the sample size calculation for proportion, sample sizes of 1075, 306 and 164 for humans, dogs and cats, respectively, were calculated.

Data collection

The study purpose and protocol was explained to all individuals and written informed consent was signed by each participant prior to study entrance. Sociodemographic data, including age (categorized into eight groups: 2–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69 and ≥ 70 y), sex, place of residence (urban or rural), number of family members (1–2=low, 3–5=medium and ≥ 6 =high), economic status according to information provided by the Statistical Center of Iran (poor, moderate, good), occupation (high occupational exposure risk vs low exposure risk) and education level (illiterate, elementary, secondary, college) were collected through a structured questionnaire. Risk factors and habits that could be associated with *Toxocara* seropositivity were also obtained through another questionnaire. Information regarding possible clinical manifestations, including asthma, shortness of breath, visual impairment, blurred vision, abdominal pain, muscle weakness and epilepsy, were collected by referring to the personal health records that were previously completed by a physician based on clinical examination and self-reports and archived in the urban and rural health centres. Information such as sex, age (≤ 1 y and >1 y), animal type (according to their function in the human society: companion dog or cat, stray dog or cat, pastoral dog and guard dog), place of residence (rural or urban) and whether the individual had taken anthelmintics during the last 2 months were collected by interviewing the animal owner.

Human sampling and laboratory tests

A 5 ml venous blood sample was taken from each participant and 2 ml was transferred into an ethylenediaminetetraacetic acid (EDTA) tube for complete blood count using an xs-500i haematology analyser (Sysmex, Kobe, Japan). The absolute number of eosinophils was then calculated by multiplying the percentage of eosinophils in the number of white blood cells per microlitre unit. The 3 ml EDTA-free blood sample was centrifuged for 10 min at 1000 g for serum separation and the serum sample was kept at –20°C until serological testing. The *Toxocara* immunoglobulin G ELISA kit (NovaTec Immundiagnostica, Dietzenbach, Germany) with a diagnostic sensitivity of 96.9% and specificity of 98.6% was used according to the manufacturer's instructions to detect antibodies against TES antigens.

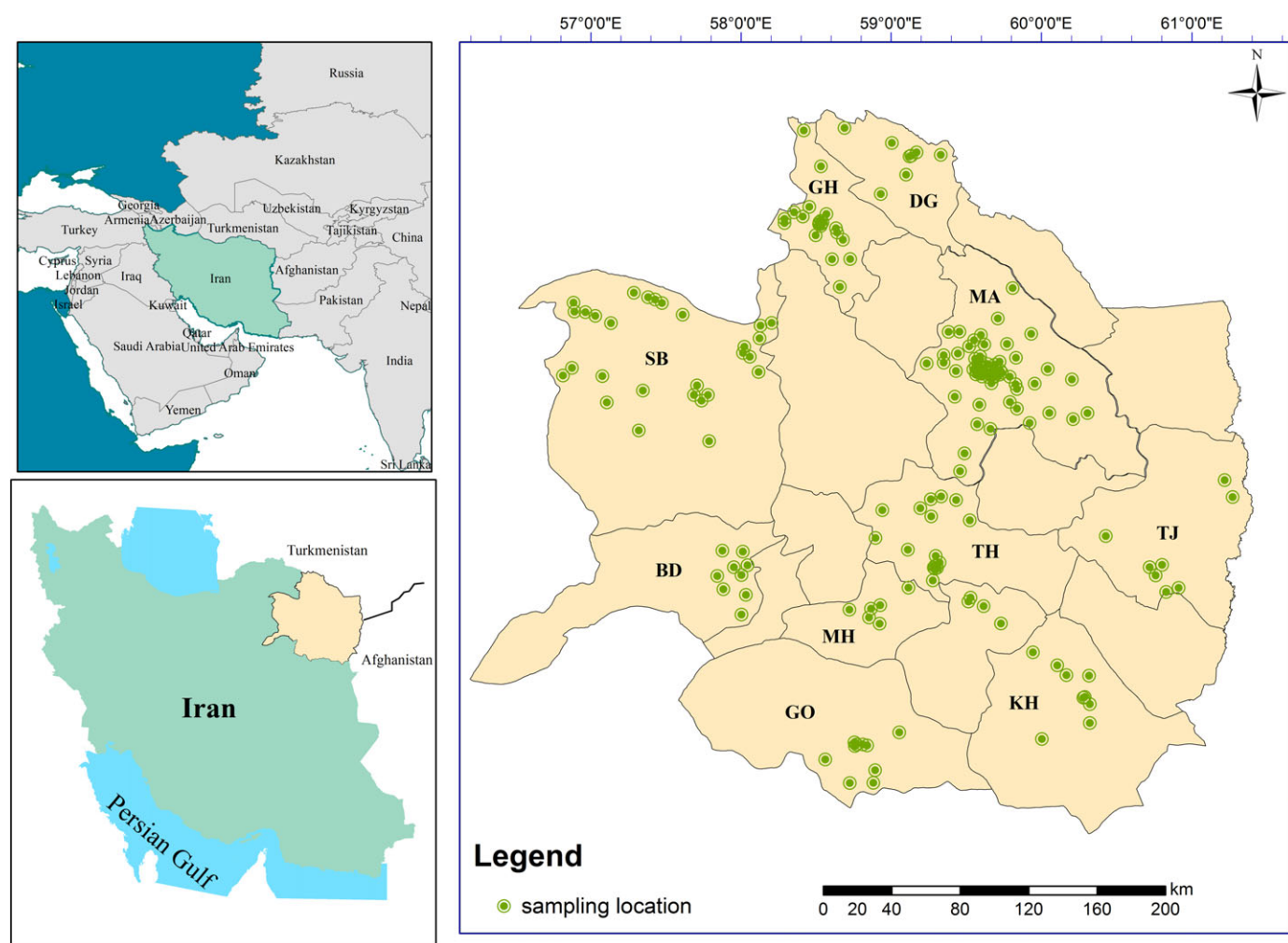


Figure 1. Geographic location of Iran and Khorasan Razavi province and distribution of sampling sites in different counties. BD: Bardaskan; DG: Dargaz; GH: Ghuchan; GO: Gonabad; KH: Khaf; MA: Mashhad; MH: Mahvelat; SB: Sabzevar; TH: Torbat Heydariyeh; TJ: Torbat Jam.

Accordingly, samples with values >11 IU/ml were considered positive, <9 IU/ml as negative and 9–11 IU/ml as suspected. All positive and suspected samples were rechecked to ensure the results accuracy. Only values >11 IU/ml were considered as seropositive and further analysed.

Animal sampling and laboratory testing

Freshly excreted stool samples were collected in a plastic sampling container that was prefilled halfway with 10% neutral buffered formalin. Stool specimens were kept at 4°C in the refrigerator until testing. The formalin–ether concentration method was used for analysing all samples.²⁵ In the next step, a faeces–formalin mixture was filtered through dual-layer gauze and 7 ml of the suspension was transferred into a centrifuge tube where 3 ml of ether was added. The tubes were shaken vigorously for 1 min and centrifuged at 2000 g for 2 min. By ringing the applicator to the inner surface of the tube, the strong adhesion of the debris plug was loosened between the two liquid phases and the top three layers were carefully

decanted. In order to prepare the smear, a drop of sediment was placed on a glass slide using a Pasteur pipette and was covered with a coverslip. To detect *Toxocara* eggs, all slides were examined by an expert microscopist using the diagnostic key and $100\times$ magnification.

Statistical analysis

Encrypted data were entered into SPSS software, version 13.0 (SPSS, Chicago, IL, USA). Descriptive statistics were used to determine the variables of the study. The χ^2 test and logistic regression test were used for analysis of categorical variables, including age categories, sex, place of residence, number of family members, socio-economic status, occupation, education level and clinical manifestations. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for sociodemographic factors. Independent-samples *t*-test was used for continuous variables in seropositive and seronegative individuals. The multiple regression model was used to compare dependent and independent variables with a probability <0.2 . The association

between possible risk factors and the outcome was determined by χ^2 test and logistic regression models with covariate adjustment. Adjusted ORs (aORs) with 95% CIs were used to evaluate the degree of dependence and a probability <0.05 was used for reporting the statistical effect on the dependent variable in multivariable analysis. Frequency distribution, percentage and χ^2 test were used to clarify the association between independent (including sex, age, place of residence, animal type and anthelmintic consumption) and dependent variables in animals.

Results

Humans: prevalence, risk factors and clinical symptoms

A total of 1150 individuals were studied; 537 (46.7%) males and 613 (53.3%) females with an average age of 31.9 ± 18.3 y (range 2–75). The overall seroprevalence of toxocariasis in the study

population was 7.2% (95% CI 5.7–8.7). The highest prevalence of anti-*Toxocara* antibodies was detected in individuals <9 y of age (11.3% [95% CI 3.7 to 32.5]) and those with a high risk of exposure to parasite eggs (13% [95% CI 1.8 to 4.8]). Age ($p<0.004$), place of residence ($p<0.001$), occupational exposure to infection ($p<0.001$) and literacy level ($p=0.001$) had a significant association with infection. However, no significant association was found between infection and sex ($p=0.23$), county of residence ($p=0.98$) and economic status ($p=0.77$) (Table 1, Figure 2).

The frequency of clinical manifestations such as asthma ($p<0.002$), shortness of breath ($p<0.006$), decreased vision ($p=0.021$), abdominal pain ($p<0.001$), epilepsy ($p<0.035$) and eosinophilia ($p<0.001$) was significantly higher in serologically positive individuals compared with negative individuals. However, there was no significant association between infection and blurred vision ($p=0.072$) or muscle weakness ($p=0.150$) (Table 2). A significant difference in the scores for absolute

Table 1. Prevalence of anti-*Toxocara* antibodies in the general population of Khorasan Razavi in addition to sociodemographic factors with crude and adjusted ORs and 95% CIs

Characteristic	Total, n (%)	Positive, n (%)	Crude OR (95% CI)	Crude p-value	Adjusted OR (95% CI)	Adjusted p-value
Sex						
Female	613 (53.3)	38 (6.2)	1.0		NA	NA
Male	537 (46.7)	45 (8.4)	1.3 (0.8 to 2.0)	NS	NA	NA
Age (years)						
2–9	130 (11.3)	26 (20)	11.0 (3.7 to 32.5)	0.001	8.6 (1.4 to 53)	0.019
10–19	179 (15.6)	15 (8.4)	4.0 (1.31 to 12.4)	0.015	1.4 (0.2 to 8.6)	NS
20–29	315 (27.4)	14 (4.4)	2.0 (0.6 to 6.3)	NS	0.7 (0.1 to 4.9)	NS
30–39	181 (15.7)	4 (2.2)	1.2 (0.4 to 2.8)	NS	0.3 (0.08 to 2.1)	NS
40–49	95 (8.3)	6 (6.3)	2.9 (0.8 to 10.8)	0.097	0.8 (0.1 to 5.4)	NS
50–59	137 (11.9)	8 (5.8)	2.7 (0.8 to 9.3)	NS	0.5 (0.9 to 3.2)	NS
60–69	88 (7.6)	8 (9.1)	4.4 (1.2 to 15.1)	0.018	1.1 (0.1 to 6.1)	NS
≥ 70	25 (2.2)	2 (8)	1.0		1.0	
Residence area						
Urban	490 (42.6)	20 (4.1)	1.0		1.0	
Rural	660 (57.4)	63 (9.6)	2.6 (1.5 to 4.5)	0.001	1.9 (1.1 to 3.8)	0.048
Occupation						
Low exposure	845 (77.4)	40 (4.7)	1.0		1.0	
High exposure	247 (22.6)	32 (13)	2.99 (1.8 to 4.8)	0.001	3.5 (1.8 to 6.7)	0.001
Number of family members						
Low	130 (11.3)	8 (6.2)	1.0		NA	NA
Medium	806 (70.1)	57 (7.1)	1.16 (0.54 to 2.49)	NS	NA	NA
High	214 (18.6)	18 (8.4)	1.40 (0.59 to 3.31)	NS	NA	NA
Education level						
College	128 (11.1)	5 (3.9)	1.0		1.0	
Secondary	468 (40.7)	23 (4.9)	0.6 (0.2 to 1.9)	NS	0.7 (0.2 to 2.3)	NS
Elementary	336 (29.2)	33 (9.9)	2.2 (1.2 to 4.2)	0.009	0.7 (0.2 to 2.8)	NS
Illiterate	218 (19)	22 (10.1)	2.3 (1.3 to 4.0)	0.002	1.6 (0.4 to 6.2)	NS
Economic status						
High	25 (2.3)	1 (4)	1.0		NA	NA
Medium	157 (14.4)	53 (7.2)	1.8 (0.2 to 14.6)	NS	NA	NA
Poor	909 (83.3)	27 (8.1)	1.9 (0.2 to 14.7)	NS	NA	NA

NA: not applicable; NS: not significant.

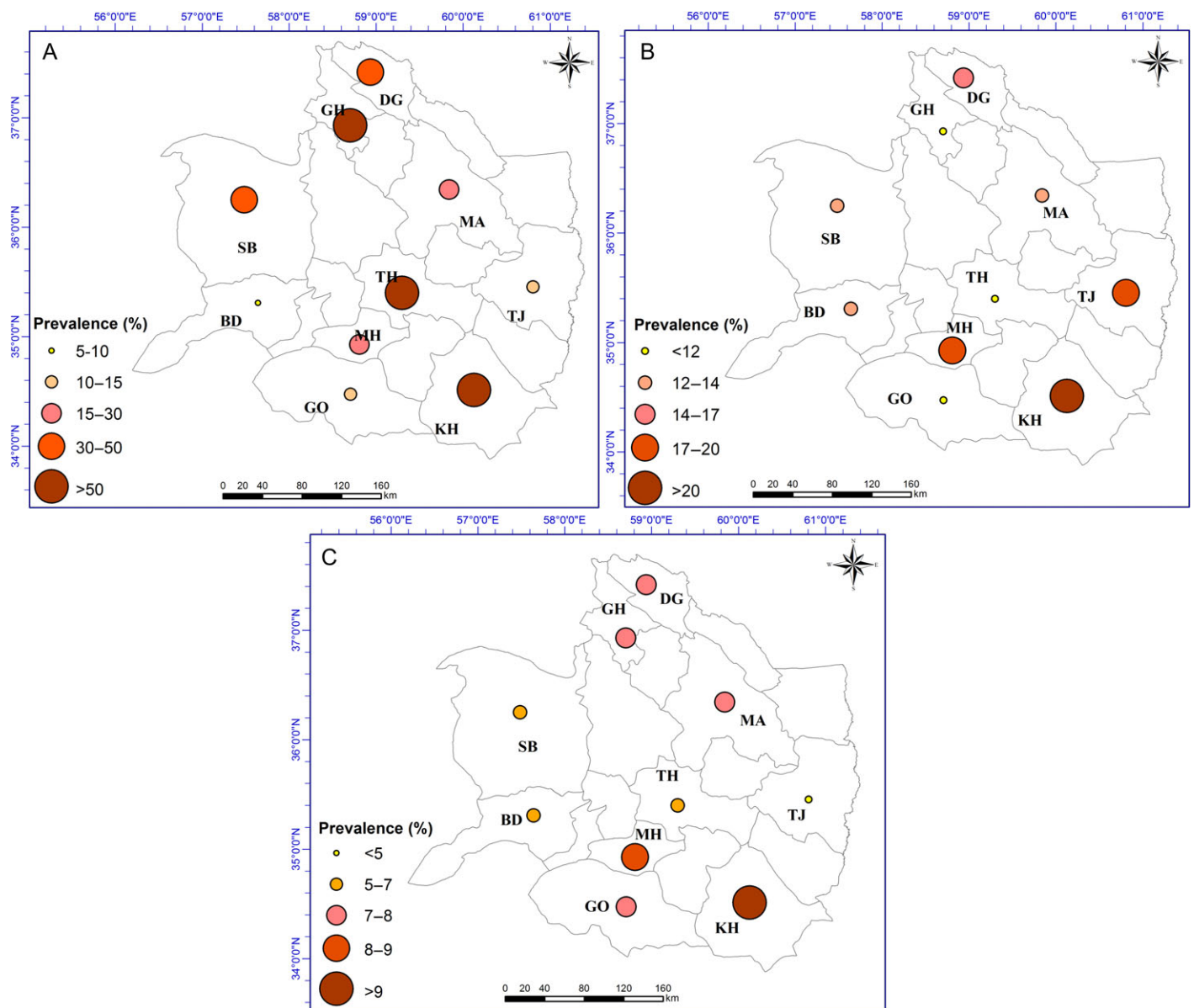


Figure 2. The prevalence of toxocarosis in different counties of Khorasan Razavi province. (A) The prevalence of infection in dogs. (B) The prevalence of infection in cats. (C) The prevalence of infection in humans. BD: Bardaskan; DG: Dargaz; GH: Ghuchan; GO: Gonabad; KH: Khaf; MA: Mashhad; MH: Mahvelat; SB: Sabzevar; TH: Torbat Heydariyeh; TJ: Torbat Jam.

eosinophil count was observed between seronegative (mean=348.7 [standard deviation {SD}=178.4]) and seropositive individuals (mean=1224.1 [SD=897.3]; $t[82.5]=8.8$, $p \leq 0.001$). A total of 40 of 69 (57.9% [95% CI 46.3 to 69.6]) patients with suspected toxocarosis (having at least one clinical symptom along with eosinophilia) were serologically positive to *Toxocara* sp. antigens, whereas only 43 of 1081 (4.1% [95% CI 2.8 to 5.1]) non-suspected individuals were seropositive ($p < 0.001$). In multivariable logistic regression analyses, contact with dogs or cats (aOR 2.9 [95% CI 1.4 to 6.1], $p=0.003$), contact with soil (aOR 2.6 [95% CI 1.5 to 3.9], $p \leq 0.001$), history of geophagy (aOR 4.3 [95% CI 2.1 to 8.6], $p=0.001$) and consumption of raw or undercooked liver (aOR 3.1 [95% CI 1.7 to 5.3],

$p=0.001$) had a significant association with *Toxocara* seropositivity (Table 3).

Among dietary and hygienic habits, high and moderate contact with dogs or cats, high consumption of raw or undercooked liver, high and moderate consumption of spring and qanat water for drinking, high and average daily contact with soil and very poor and poor hand washing were significantly associated with *Toxocara* seropositivity (Table 4).

Animals: prevalence and related factors

A total of 538 dogs and 236 cats, mainly of mixed breeds, were recruited in this study. *Toxocara* sp. eggs were detected in

Table 2. The frequency of clinical manifestations and eosinophilia in serologically positive and negative individuals

Feature	Total, n (%)	Positive, n (%)	OR (95% CI)	p-Value
Asthma				
No	1116 (97)	75 (6.7)	1.0	
Yes	34 (3)	8 (23.5)	4.2 (1.8 to 9.7)	0.001
Shortness of breath				
No	1050 (91.3)	66 (6.3)	1.0	
Yes	40 (3.5)	14 (14)	2.3 (1.2 to 4.2)	0.008
Blurred vision				
No	1102 (95.8)	76 (6.9)	1.0	
Yes	48 (4.2)	7 (14.6)	2.3 (0.9 to 5.3)	NS
Decreased vision				
No	1110 (96.5)	76 (6.8)	1.0	
Yes	40 (3.5)	7 (17.5)	2.8 (1.2 to 6.7)	0.014
Abdominal pain				
No	992 (86.3)	59 (5.9)	1.0	
Yes	158 (13.7)	12 (10.5)	2.83 (1.70 to 4.70)	0.001
Muscle weakness				
No	1036 (90.1)	71 (6.9)	1.0	
Yes	114 (9.9)	12 (10.5)	1.59 (0.83 to 3.04)	NS
Epilepsy				
No	1132 (98.4)	79 (7)	1.0	
Yes	18 (1.6)	4 (22.2)	3.80 (1.25 to 11.84)	0.021
Eosinophilia				
No	1028 (89.4)	26 (2.5)	1.0	
Yes	122 (10.6)	57 (46.7)	33.7 (19.9 to 57.2)	0.001

NS: not significant.

15.1% (95% CI 12.1 to 18.1) of dogs and 34.7% (95% CI 28.6 to 40.8) of cats. The highest rate of *Toxocara* infection was identified in stray dogs (26.7%) and cats (38.8%), in animals >1 y of age (55.9% of dogs, 75.9% of cats) and animals not treated with anthelmintics during the 2 months prior to sampling (23% of dogs, 39.3% of cats). The incidence of infection in stray cats (38.8% [95% CI 31.9 to 45.6]) was 2.5 times higher than that of companion cats (15.0% [95% CI 3.9 to 26.1]). There was no significant difference in the prevalence of infection among different counties ($p=0.150$) (Figure 2). The frequency of infection in rural dogs was significantly higher than that of urban dogs ($p=0.010$), but no such difference was observed between urban and rural cats ($p=0.340$) (Table 5).

Discussion

Toxocarasis is a neglected parasitic disease that can cause serious health problems. This study was conducted because of the lack of epidemiological data on this infection in northeast Iran. The similar seroprevalence rates identified by Baghani et al.²⁶ in Tehran (5.6% [21/374]) and the current study (7.2% [83/1150]) may be due to the similar semi-arid climate in these two

regions. On the other hand, the high prevalence of exposure to infection in the general population of northern Iran²⁷ (23.5% [148/630]) may be due to the humid climate, moist soil and suitable temperature for development of *Toxocara* eggs.²⁷ The exposure rate to *Toxocara* sp. observed in the present study is in agreement with others performed in Austria²⁸ (6.3% [66/1046]), Australia²⁹ (7% [22/314]), Sweden³⁰ (7% [16/323]) and The Netherlands³¹ (8.0% [295/3683]). This similarity may be due to the similar climatic conditions, as Australia has an arid and semi-arid climate and the other countries have cold climates. These countries also have relatively similar economic status that may play an important role in *Toxocara* epidemiology via its impact on public health and sanitation.

The prevalence of anti-*Toxocara* antibodies in children and adults of developing countries such as Cuba³² and Vietnam³³ has been reported as 40.1% (384/958) and 45.2% (34965/77356), respectively, while the seroprevalence rates in developed countries such as Spain³⁴ and Japan¹² have been reported as 2.1% (10/467) and 1.6% (52/3277), respectively. The higher seroprevalence rates in Cuba and Vietnam may be due to the temperature and high humidity of these regions that favour the embryonation of *Toxocara* ova in the soil.¹¹ Economic status may also affect the epidemiology compared with countries with economic difficulties such as Cuba. Nevertheless, the sensitivity and specificity of the ELISA method varies and strongly depends on the target antigens and antibodies chosen by the manufacturer. Due to the lack of an alternative confirmation method, such as western blot, and the possible cross-reactivity with other infective agents, the few false-positive results cannot be ignored.

Among sociodemographic factors, younger age (aOR 8.6, $p=0.019$), living in a rural area (aOR 1.9, $p=0.048$) and occupations with high exposure risk (aOR 3.5, $p=0.001$) had a higher seroprevalence rate. These results are consistent with the studies by Alavi et al.³⁵ (Iran), Lassen et al.³⁶ (Estonia), Liu et al.⁷ (United States) and Deutz et al.³⁷ (Austria), but contrary to the results of Aghamolaie et al.²⁷ and Baghani et al.²⁶ (Iran) and Mughini-Gras et al.³¹ (The Netherlands). In the present study, frequent contact with soil, a history of geophagy and eating raw or undercooked liver were risk factors for toxocarasis. Aghamolaie et al.²⁷ (Iran), Mughini-Gras et al.³¹ (The Netherlands) and Negri et al.³⁸ (Brazil) reported that contact with soil is associated with *Toxocara* seroprevalence, whereas Khoshnood et al.³⁹ (Iran), Baghani et al.²⁶ (Iran) and Fu et al.¹⁵ (Marshall Islands) did not come to this conclusion. Although Cassenote et al.⁴⁰ (Brazil) found a significant relationship between infection and hand washing, this association was not seen by Martinez et al.⁴¹ (Venezuela). Frequent contact with soil and unrinsed hands increase the possibility of exposure to *Toxocara* sp. eggs.^{40,42,43}

Similar to other studies,^{15,41,44,45} in the present study a significant association was found between contact with dogs or cats and *Toxocara* seroprevalence; however, this finding is not supported by other surveys.^{26,35,46} The present study showed that individuals with a history of geophagy are 4.3 times (95% CI 2.1 to 8.6; $p\leq0.001$) more likely to have been exposed to *Toxocara* than non-geophagic cases. Several epidemiological studies from Iran,^{21,35} India,⁴⁵ The Netherlands³¹ and Brazil⁴⁰ have shown a significant relationship between geophagy and

Table 3. Risk factors and their relationships with *T. canis* seropositivity in humans

Variable	Total, n (%)	<i>T. canis</i> seropositive, n (%)	Crude OR (95% CI)	Crude p-value	Adjusted OR (95% CI)	Adjusted p-value
Keeping dogs or cats at home						
No	805 (70.0)	30 (3.7)	1.0			
Yes	345 (30.0)	53 (15.3)	4.6 (2.9 to 7.4)	0.001	1.4 (0.5 to 3.5)	NS
Contact with dogs or cats						
No	980 (85.2)	46 (4.7)	1.0			
Yes	170 (14.8)	37 (21.7)	5.6 (3.5 to 9.0)	0.001	2.9 (1.4 to 6.1)	0.003
Feeding dogs or cats at home						
No	1010 (87.8)	62 (6.1)	1.0			
Yes	140 (12.2)	21 (15.0)	2.6 (1.5 to 4.5)	0.001	1.1 (0.5 to 2.3)	NS
Collect and dispose of dog or cat stool properly						
No	954 (82.9)	51 (5.3)	1.0			
Yes	196 (17.1)	32 (16.3)	3.4 (2.1 to 5.5)	0.001	1.2 (0.6 to 2.4)	NS
Wash hands after contact with dogs or cats						
No	1026 (89.3)	57 (5.5)	1.0			
Yes	123 (10.7)	26 (21.1)	4.5 (2.7 to 7.5)	0.001	1.7 (0.8 to 3.5)	NS
Contact with soil						
No	782 (68.2)	38 (4.9)	1.0			
Yes	364 (31.8)	45 (12.4)	2.8 (1.7 to 4.3)	0.001	2.6 (1.5 to 3.9)	0.001
Dirty courtyard						
No	745 (65.6)	39 (5.2)	1.0			
Yes	390 (34.4)	43 (11.0)	2.2 (1.4 to 3.5)	0.001	1.4 (0.8 to 2.4)	NS
History of geophagy						
No	1035 (90.9)	61 (5.9)	1.0			
Yes	104 (9.1)	22 (21.2)	4.2 (2.5 to 7.3)	0.001	4.3 (2.1 to 8.6)	0.001
History of nail biting						
No	1097 (96.4)	73 (6.7)	1.0			
Yes	41 (3.6)	9 (22.0)	3.9 (1.8 to 8.5)	0.001	1.7 (0.6 to 4.6)	NS
Eating grilled meat						
No	786 (68.6)	53 (6.7)	1.0			
Yes	360 (31.4)	29 (8.1)	0.8 (0.5 to 1.3)	NS	NA	NA
Consumption of raw or undercooked liver						
No	941 (82.8)	55 (5.8)	1.0			
Yes	196 (17.2)	28 (14.3)	2.6 (1.6 to 4.3)	0.001	3.1 (1.7 to 5.3)	0.001
Wear gloves when chopping meat						
No	1022 (90.0)	61 (6.0)	1.0			
Yes	127 (11.0)	22 (17.3)	1.5 (0.6 to 4.1)	NS	NA	NA
Wash the knife after cutting the meat						
No	582 (51.2)	48 (8.2)	1.0			
Yes	555 (48.8)	35 (6.3)	0.7 (0.4 to 1.1)	NS	NA	NA
Wash vegetables and fruits properly						
No	1050 (91.3)	79 (7.5)	1.0			
Yes	100 (8.7)	4 (4.0)	0.5 (0.1 to 1.4)	NS	NA	NA

NA: not applicable; NS: not significant.

the presence of anti-*Toxocara* antibodies in humans. Yet this has not been seen in other studies from Iran,^{39,47} Brazil³⁸ and Venezuela.⁴¹ According to another study, toxocariasis is the most common parasitic disease associated with geophagy in the United States.⁴⁸ Choi et al.⁴ (Korea) and Fan et al.⁴⁹ (Taiwan) observed no significant relationship between raw or undercooked liver consumption and *Toxocara* seroprevalence;

however, the results of Mosayebi et al.,⁴⁴ Aghamolaie et al.²⁷ (Iran) and Negri et al.³⁸ (Brazil) do not support this finding. According to the study by Ahn et al.⁵⁰ (Korea), >80% (OR 14.9) of patients with ocular toxocariasis have a history of eating raw liver. Accordingly, some studies^{21,45} have shown a significant association between the source of water and the seroprevalence of *Toxocara*, whereas others^{27,41,46} have not reported such

Table 4. The dietary and hygiene habits and their association with *T. canis* seropositivity in the study population

Variable	Total, n (%)	<i>T. canis</i> seropositive, n (%)	OR (95% CI)	p-Value
Contact with dogs and cats				
High	17 (10.0)	8 (47.1)	7.7 (2.2 to 26.5)	0.001
Medium	34 (20.0)	11 (32.4)	4.1 (1.4 to 12.0)	0.008
Low	51 (30.0)	11 (21.6)	2.3 (0.8 to 6.7)	NS
Very low	68 (40)	7 (10.3)	1.0	
Raw vegetable consumption				
High	283 (25)	21 (7.4)	0.7 (0.3 to 1.4)	NS
Medium	436 (38.5)	21 (4.8)	0.5 (0.2 to 0.9)	NS
Low	245 (21.7)	24 (9.8)	1.1 (0.5 to 2.0)	NS
Very low	168 (14.8)	16 (9.5)	1.0	
Meat consumption				
High	101 (8.9)	9 (8.9)	1.2 (0.4 to 3.0)	NS
Medium	632 (55.8)	38 (6)	0.7 (0.3 to 1.6)	NS
Low	268 (23.6)	25 (9.3)	1.2 (0.5 to 2.7)	NS
Very low	133 (11.7)	10 (7.5)	1.0	
Amount of raw or undercooked liver consumed				
High	57 (29.1)	8 (14.0)	4.8 (1.4 to 15.6)	0.009
Medium	57 (29.1)	2 (10.5)	2.4 (0.6 to 8.4)	NS
Low	19 (9.7)	4 (6.3)	1.7 (0.2 to 10.3)	NS
Very low	63 (32.1)	28 (14.3)	1.0	
Amount of spring and aqueduct water used for drinking				
High	41 (3.6)	6 (14.6)	2.7 (1.1 to 6.8)	0.034
Medium	128 (11.3)	15 (11.7)	2.1 (1.1 to 3.9)	0.020
Low	305 (27)	23 (7.5)	1.2 (0.7 to 2.2)	NS
Very low	658 (58.1)	39 (5.9)	1.0	
Daily contact with soil				
High	203 (17.8)	29 (14.3)	7.6 (3.6 to 15.9)	0.001
Medium	258 (22.7)	37 (14.3)	7.6 (3.7 to 15.7)	0.001
Low	210 (18.4)	7 (3.3)	1.5 (0.5 to 4.2)	NS
Very low	468 (41.1)	10 (2.1)	1.0	
Amount of hand washing after contact with soil				
High	838 (74)	43 (5.1)	1.0	
Medium	181 (16)	15 (8.3)	1.6 (0.9 to 3.0)	NS
poor	87 (7.7)	15 (17.2)	3.8 (2.0–7.2)	0.001
Very poor	26 (2.3)	10 (38.5)	11.5 (4.9–26.9)	0.001

NS: not significant.

an association. In the current study, a part of the collected data on the socio-economic status, risk factors, personal habits and the animals were self-reported and not verified by independent methods, therefore information bias was inevitable. Moreover, although toxocariasis risk factors were considered to a great extent, not all may have been identified and these factors should be considered in future studies.

Clinical manifestation analysis revealed that subjects with asthma, shortness of breath, decreased vision, abdominal pain and epilepsy were approximately 3.5, 2.2, 2.5, 1.7 and 3.2 times, respectively, more likely to have anti-*Toxocara* antibodies compared to healthy participants. In a recently published meta-analysis, children who were exposed to *Toxocara* were more likely to develop asthma than non-exposed children.²² In the

present study, a significant relationship was found between blurred vision and the presence of anti-*Toxocara* antibodies. The findings of Martinez et al.⁴¹ (in Venezuela) are contrary to ours, probably because they only studied children. Moreover, abdominal pain has been reported as one of the most common non-specific symptoms of covert toxocariasis in children and our results highlighted a significant association between abdominal pain and infection in both children and adults.⁸ In support of the findings of the present study, several studies have shown the possible role of toxocariasis in the incidence of epilepsy.⁵¹ We also found a significant association between eosinophilia and *Toxocara* infection (46.7% in seropositive and 2.5% in sero-negative individuals), similar to other studies.^{4,23,33,35} We do not have follow-up of suspected persons to determine definite

Table 5. Prevalence of *Toxocara* infection in dogs and cats according to sex, age, place of residence, type and anthelmintic therapy

Characteristic	Dogs			Cats		
	Total, n (%)	Infection, n (%)	p-Value	Total, n (%)	Infection, n (%)	p-Value
Sex						
Male	455 (84.6)	64 (14.1)	NS	152 (64.4)	54 (35.5)	NS
Female	83 (15.4)	17 (20.5)		84 (35.6)	28 (33.3)	
Age (years)						
≤1	111 (21.4)	62 (55.9)	0.001	79 (36.4)	60 (75.9)	0.001
>1	408 (78.6)	17 (4.2)		138 (63.6)	15 (10.9)	
Place of residence						
Urban	102 (19)	7 (6.9)	0.010	200 (84.7)	72 (36.0)	NS
Rural	436 (81)	74 (17.0)		36 (15.3)	10 (27.8)	
Anthelmintic consumption during the last 2 months						
No	348 (66.0)	80 (23.0)	0.001	201 (85.2)	79 (39.3)	0.001
Yes	180 (34.0)	0.0 (0.0)		35 (14.8)	3 (8.6)	
Animal type ^a						
Companion	53 (9.8)	2 (3.8)	0.001	40 (16.9)	6 (15.0)	0.004
Pastoral	170 (31.6)	20 (11.8)		NA	NA	
Guard	150 (27.9)	15 (10.0)		NA	NA	
Stray	165 (30.7)	44 (26.7)		196 (83.1)	76 (38.8)	

^aThe type of animal according to its function or role in human society.

NA: not available; NS: not significant.

Toxocara infection. Therefore designing a case-control or longitudinal cohort study is recommended.

According to our results, there seems to be no difference between the overall prevalence of infection in dogs in Khorasan Razavi province (15.0%) and the average prevalence rate of infection in dogs throughout the country (12.3%). However, the overall prevalence rate in cats was higher (34.7%) than the average prevalence rate throughout the country (26.2%).⁵² Based on previous studies, the mean prevalence of *Toxocara* in stray dogs and cats in Mashhad, the capital of Khorasan Razavi province, is 19.5% and 28.8%, respectively.²⁴ These prevalences were reported as 1.4–30.5% and 8–76% in European countries.⁵³ The higher prevalence of toxocariasis in cats compared with dogs may be due to the lower resistance of adult cats to reinfection; moreover, the transmammary route of transmission in kittens and differences in ecology, food sources and eating habits may be other possible explanations.^{43,53} The present study confirms the results of other studies showing no correlation between sex and the prevalence of infection in dogs and cats. Similarly, the infection rate of puppies and kittens was higher than in adult dogs and cats. It is worth noting that the sensitivity of younger animals to infection and transmission of toxocariasis via infected mother's milk can lead to serious infections.^{24,54} According to our results, the infection rate in rural dogs was higher than that in urban dogs ($p=0.01$), while no significant difference was observed between urban and rural cats ($p=0.34$). This may be due to the greater population of stray dogs, improper collection and disposal of animal faeces or possibly the greater levels of soil contamination in rural areas.^{55,56}

On the other hand, the similar eating habits of urban and rural cats and the lack of surveillance programs in stray cats are possible reasons for the similar prevalence rates of infection among them.⁵³ Since *Toxocara* sp. is susceptible to common anthelmintics, one of the main strategies for disease prevention is treatment of infected adult animals, which can result in reduced contamination in puppies, kittens and the environment.⁵³ The close involvement of dogs and cats in everyday life can lead to increased exposure to infectious eggs due to high levels of environmental contamination. The high similarity of *T. canis* and *T. cati* antigens is the basis for the production of current diagnostic kits, whereas certain differences in antigenic molecules is inevitable. Thus, with respect to the high prevalence of infection in cats in this study, some truly positive cases in humans may have been missed due to the lack of a *T. cati* diagnostic kit.

Conclusions

Toxocariasis is common in humans, dogs and cats in northeast Iran. Stray dogs and cats, particularly those <1 y old that do not receive anthelmintics can potentially affect the transmission of infection to humans. *Toxocara* seropositivity in humans may be associated with manifestations such as asthma, shortness of breath, visual impairment, abdominal pain, eosinophilia and epilepsy and therefore is an issue of concern for public health. Contact with dogs and cats, repeated contact with soil, a history of geophagy, lack of hand washing after soil contact and raw or

undercooked liver consumption are the main risk factors associated with toxocariasis seropositivity in the human population. Since our findings show that northeast Iran is endemic for *Toxocara* sp., implementation of preventive measures such as public education, routine faecal examinations and chemotherapeutic treatment of animals is highly recommended.

Authors' contributions: MMR and SHH conceived the study. MMR, SHH and MA designed the study protocol. MRR and AE performed the faecal experiments. SH carried out the immunoassays and analysis and interpretation of the data. MA analysed the data. MRR, SHH and MA prepared the draft manuscript. All authors read and approved the final manuscript. MMR and SHH are guarantors of the paper.

Acknowledgements: The authors wish to especially thank the staff of the health care centres of the studied cities and villages who helped us collect human blood samples. We are grateful to all those who participated in this study and collaborated in sampling and data collection. We also appreciate the dog and cat owners for their kind cooperation in this study.

Funding: This study, as part of a PhD dissertation, was supported by the Council of Research, Isfahan University of Medical Sciences, Isfahan, Iran (396645).

Competing interests: None declared.

Ethical approval: The research protocol was submitted and approved by the Ethics Committee on Biomedical Research of Isfahan University of Medical Sciences, Isfahan, Iran (IR.MUI.REC.1396.3.645).

References

- Macpherson CN. The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. *Int J Parasitol.* 2013; 43(12–13):999–1008.
- Glickman LT, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiol Rev.* 1981;3:230–50.
- Lee AC, Schantz PM, Kazacos KR, et al. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends Parasitol.* 2010;26(4):155–61.
- Choi D, Lim JH, Choi DC, et al. Transmission of *Toxocara canis* via ingestion of raw cow liver: a cross-sectional study in healthy adults. *Korean J Parasitol.* 2012;50(1):23.
- Stürchler D, Weiss N, Gassner M. Transmission of toxocariasis. *J Infect Dis.* 1990;162(2):571.
- Salem G, Schantz P. Toxocaral visceral larva migrans after ingestion of raw lamb liver. *Clin Infect Dis.* 1992;15(4):743–4.
- Liu EW, Chastain HM, Shin SH, et al. Seroprevalence of antibodies to *Toxocara* species in the United States and associated risk factors, 2011–2014. *Clin Infect Dis.* 2017;66(2):206–12.
- Ma G, Holland CV, Wang T, et al. Human toxocariasis. *Lancet Infect Dis.* 2018;18(1):e14–24.
- Magnaval J-F, Glickman LT, Dorchie P, et al. Highlights of human toxocariasis. *Korean J Parasitol.* 2001;39(1):1.
- Moreira G, de Lima Telmo P, Mendonça M, et al. Human toxocariasis: current advances in diagnostics, treatment, and interventions. *Trends Parasitol.* 2014;30(9):456–64.
- Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clin Microbiol Rev.* 2003;16(2):265–72.
- Kondo K. Sero-epidemiological investigation of toxocariasis in Asian area. In: Yamaguchi T, Araki T, Chen ER (eds.). *The 5th Asian-Pacific Congress for Parasitic Zoonoses*, 1998. Taipei: Organizing Committee Asian-Pacific Congress for Parasitic Zoonoses, 1998; 65–70.
- Stensvold CR, Skov J, Møller LN, et al. Seroprevalence of human toxocariasis in Denmark. *Clin Vaccine Immunol.* 2009;16(9):1372–3.
- Rai SK, Uga S, Ono K, et al. Seroepidemiological study of *Toxocara* infection in Nepal. *Southeast Asian J Trop Med Public Health.* 1996; 27:286–90.
- Fu CJ, Chuang TW, Lin HS, et al. Seroepidemiology of *Toxocara canis* infection among primary schoolchildren in the capital area of the Republic of the Marshall Islands. *BMC Infect Dis.* 2014;14:261.
- Traub RJ, Robertson ID, Irwin PJ, et al. Canine gastrointestinal parasitic zoonoses in India. *Trends Parasitol.* 2005;21(1):42–8.
- Sowemimo OA. Prevalence and intensity of *Toxocara canis* (Werner, 1782) in dogs and its potential public health significance in Ile-Ife, Nigeria. *J Helminthol.* 2007;81(4):433–8.
- Dai R, Li Z, Li F, et al. Severe infection of adult dogs with helminths in Hunan Province, China poses significant public health concerns. *Vet Parasitol.* 2009;160(3–4):348–50.
- Waap H, Gomes J, Nunes T. Parasite communities in stray cat populations from Lisbon, Portugal. *J Helminthol.* 2014;88(4):389–95.
- Hosseini-Safa A, Mousavi SM, Badorani MB, et al. Seroepidemiology of toxocariasis in children (5–15 yr old) referred to the pediatric clinic of Imam Hossein Hospital, Isfahan, Iran. *Iran J Parasitol.* 2015;10(4):632–7.
- Garedaghi Y. Seroprevalence of toxocariasis in children in East-Azerbaijan Province, Iran. *Cukurova Med J.* 2013;38(4):581–6.
- Aghaei S, Riahi SM, Rostami A, et al. *Toxocara* spp. infection and risk of childhood asthma: a systematic review and meta-analysis. *Acta Trop.* 2018;182:298–304.
- Won KY, Kruszon-Moran D, Schantz PM, et al. National seroprevalence and risk factors for zoonotic *Toxocara* spp. infection. *Am J Trop Med Hyg.* 2008;79(4):552–7.
- Zibaei M, Sadjjadi SM. Trend of toxocariasis in Iran: a review on human and animal dimensions. *Iran J Vet Res.* 2017;18(4):233–42.
- Ridley D, Hawgood B. The value of formol-ether concentration of faecal cysts and ova. *J Clin Pathol.* 1956;9(1):74–6.
- Baghani Z, Khazan H, Sohrabi MR, et al. Seroprevalence of *Toxocara* infection among healthy individuals referred to the medical center laboratories in Tehran City, capital of Iran. *Novelty BioMed.* 2018;6(2):68–73.
- Aghamolaie S, Seyyedtabaei SJ, Behniafar H, et al. Seroepidemiology, modifiable risk factors and clinical symptoms of *Toxocara* spp. infection in northern Iran. *Trans R Soc Trop Med Hyg.* 2018;113(3):116–122.
- Poepl W, Herkner H, Tobudic S, et al. Exposure to *Echinococcus multilocularis*, *Toxocara canis*, and *Toxocara cati* in Austria: a nationwide cross-sectional seroprevalence study. *Vector Borne Zoonotic Dis.* 2013;13(11):798–803.
- Nicholas WL, Stewart AC, Walker JC. Toxocariasis: a serological survey of blood donors in the Australian Capital Territory together with observations on the risks of infection. *Trans R Soc Trop Med Hyg.* 1986;80(2):217–21.

- 30 Ljungström I, van Knapen F. An epidemiological and serological study of *Toxocara* infection in Sweden. *Scand J Infect Dis*. 1989;21(1):87–93.
- 31 Mughini-Gras L, Harms M, van Pelt W, et al. Seroepidemiology of human *Toxocara* and *Ascaris* infections in the Netherlands. *Parasitol Res*. 2016;115(10):3779–94.
- 32 Kanobana K, Vereecken K, Junco Diaz R, et al. *Toxocara* seropositivity, atopy and asthma: a study in Cuban schoolchildren. *Trop Med Int Health*. 2013;18(4):403–6.
- 33 Nguyen T, Cheong FW, Liew JWK, et al. Seroprevalence of fascioliasis, toxocarosis, strongyloidiasis and cysticercosis in blood samples diagnosed in Medic Medical Center Laboratory, Ho Chi Minh City, Vietnam in 2012. *Parasit Vectors*. 2016;9:486.
- 34 Fenoy S, Cuellar C, Guillen J. Seroprevalence of toxocarosis in children and adults in Madrid and Tenerife, Spain. *J Helminthol*. 1996;70(2):109–13.
- 35 Alavi SM, Hosseini SA, Rahdar M, et al. Determination of seroprevalence rate of *Toxocara canis* in 6–15 years aged rural and urban school children in Ahvaz, Iran. *Jundishapur Sci Med J*. 2011;10(3):239–48.
- 36 Lassen B, Janson M, Viltrop A, et al. Serological evidence of exposure to globally relevant zoonotic parasites in the Estonian population. *PLoS One* 2016;11(10):e0164142.
- 37 Deutz A, Fuchs K, Auer H, et al. *Toxocara*-infestations in Austria: a study on the risk of infection of farmers, slaughterhouse staff, hunters and veterinarians. *Parasitol Res*. 2005;97(5):390–4.
- 38 Negri EC, Santarém VA, Rubinsky-Elefant G, et al. Anti-*Toxocara* spp. antibodies in an adult healthy population: serosurvey and risk factors in southeast Brazil. *Asian Pac J Trop Biomed*. 2013;3(3):211–6.
- 39 Khoshnood S, Pestehchian N, Hejazi SH, et al. *Toxocara* seroprevalence and associated risk factors among Ilam children, West of Iran. *Arch Pediatr Infect Dis*. 2018;7(1):e81349.
- 40 Cassenote AJ, de Abreu Lima AR, Neto JM, et al. Seroprevalence and modifiable risk factors for *Toxocara* spp. in Brazilian schoolchildren. *PLoS Negl Trop Dis*. 2014;8(5):e2830.
- 41 Martínez M, Montero J, Pineda A, et al. Epidemiological, clinical and laboratory features of toxocarosis in school children from Aragua State, Venezuela. *Trans R Soc Trop Med Hyg*. 2018;112(6):255–63.
- 42 Congdon P, Lloyd P. *Toxocara* infection in the United States: the relevance of poverty, geography and demography as risk factors, and implications for estimating county prevalence. *Int J Public Health*. 2011;56(1):15–24.
- 43 Overgaauw PA, Nederland V. Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. *Crit Rev Microbiol*. 1997;23(3):233–51.
- 44 Mosayebi M, Moini L, Hajihosseini R, et al. Detection of specific antibody reactivity to *Toxocara* larval excretory-secretory antigens in asthmatic patients (5–15 years). *Open Microbiol J*. 2016;10:162–7.
- 45 Dar Z, Tanveer S, Yattoo G, et al. Seroprevalence of toxocarosis in children in Kashmir, J&K State, India. *Iran J Parasitol*. 2008;3(4):45–50.
- 46 Sarkari B, Alirezai R, Layegh Gigloo A, et al. Seroprevalence and risk factors for *Toxocara* infection among children in a rural community in Fars province, southern Iran. *Parasite Immunol*. 2018;40(11):e12582.
- 47 Akhlaghi L, Ourmazdi H, Sarafnia A, et al. An investigation on the toxocarosis seroprevalence in children (2–12 years old) from Mahidasht area of Kermanshah Province (2003–2004). *Razi J Med Sci*. 2006;13(52):41–8.
- 48 Callahan G. Eating dirt. *Emerg Infect Dis*. 2003;9(8):1016–21.
- 49 Fan CK, Lan HS, Hung CC, et al. Seroepidemiology of *Toxocara canis* infection among mountain aboriginal adults in Taiwan. *Am J Trop Med Hyg*. 2004;71(2):216–21.
- 50 Ahn SJ, Woo SJ, Jin Y, et al. Clinical features and course of ocular toxocarosis in adults. *PLoS Negl Trop Dis*. 2014;8(6):e2938.
- 51 Luna J, Cicero CE, Rateau G, et al. Updated evidence of the association between toxocarosis and epilepsy: systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2018;12(7):e0006665.
- 52 Sarvi S, Daryani A, Sharif M, et al. Zoonotic intestinal parasites of carnivores: a systematic review in Iran. *Vet World*. 2018;11(1):58–65.
- 53 Baneth G, Thamsborg S, Otranto D, et al. Major parasitic zoonoses associated with dogs and cats in Europe. *J Comp Pathol*. 2016;155(1 Suppl 1):S54–74.
- 54 Calvete C, Lucientes J, Castillo JA, et al. Gastrointestinal helminth parasites in stray cats from the mid-Ebro Valley, Spain. *Vet Parasitol*. 1998;75(2–3):235–40.
- 55 Habluetzel A, Traldi G, Ruggieri S, et al. An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. *Vet Parasitol*. 2003;113(3–4):243–52.
- 56 García LC, Alvarez AM, Martín FS. Epidemiological studies on toxocarosis and visceral larva migrans in a zone of western Spain. *Ann Trop Med Parasitol*. 1989;83(6):615–20.