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Original Article

Seroprevalence of Canine Toxocariasis in Three Rural Areas of Fars Province, Southern Iran

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Abstract

Background: *Toxocara canis* is one of the most important causes of animal toxocariasis with global distribution. We aimed to find out the seroprevalence of toxocariasis in dogs in a rural area in Fars Province, south of Iran.

Methods: Dogs blood samples were collected from 60 dogs in three rural areas in the Sar Mashhad region, Fars Province. Dog sera were evaluated for anti-*Toxocara* antibodies by an indirect ELISA method. The association between the seropositivity and age, gender, and the sampling location were statistically evaluated.

Results: Serological assay detected anti-*Toxocara* antibodies in sera of 32 out of 60 dogs, corresponding to a seroprevalence of 53.3%. The rate of seropositivity was higher in the male dogs. The rate of seropositivity was higher in old dogs. This rate increased with increasing age, however, the association between age and *Toxocara* seropositivity was not statistically significant.

Conclusion: The high prevalence of *Toxocara* infection in dogs in the current study area confirms that infected dogs are an important source of *Toxocara* infection for their owners and people who are in close contact with these animals, especially children.

Introduction

Toxocariasis is a zoonotic parasitic infection caused mainly by *Toxocara canis* and *Toxocara cati*, which live in the small intestine of canids and felids as final hosts, respectively (1). *T. canis* infects dogs, wolves, jackals, dingoes, and red foxes (2). The adult worm lives in the dog's small intestine where it produces 200,000 eggs per day. These eggs are embryonated in the soil at a temperature of 26-30 °C and ambient humidity within 2 to 3 weeks. If the final host eats infectious eggs, they hatch in the small intestine and travel to the lungs and liver via the bloodstream. They reach the small intestine through the trachea and become an adult worm (3). In older dogs, the migration of larvae is not limited to the lungs; as they migrate to other organs such as the liver, brain, heart, and skeletal muscles. (3, 4).

Humans and other intermediate hosts get infected through contaminated soil, eating vegetables contaminated with embryonated eggs or consuming raw meat of the paratenic hosts (5). *Toxocara* causes different clinical syndromes. Larvae can migrate to the liver, kidney, and lung and cause visceral larva migrans (VLM), or enter the eye and form ocular larva migrans (OLM). The larvae may also go to the central nervous system and form neural larva migrans (6).

Toxocariasis is one of the most common helminthic infections in humans, and reports of this disease are widely seen on five continents. In Iran and the world, a high level of contamination of dogs and cats with the *Toxocara* parasite has been reported. Contaminated soil is one of the most important sources of *Toxocara* infection for humans (3). Some studies in Iran have examined soil contamination in public places such as parks, beach sand, and children's sandboxes, and the contamination rate of *Toxocara* eggs was reported to be between 3.62 and 38.67% (7,8). Choobineh et al. reported a 16% contamination rate of *Toxocara* eggs in the public parks and playgrounds of Shiraz City, capital of Fars Province (7). Abbaszadeh et al, reviewed the results of 15 studies on *Toxocara* infection in stray dogs in Iran and reported the prevalence of 1.78 to 36.61% in these animals (8).

Dog infection with *T. canis* is sometimes associated with clinical symptoms such as abdominal swelling, constipation, vomiting, diarrhea, and flatulence (3). The common method to detect *Toxocara* infection in dogs is to observe brown eggs with a porous shell in the stool sample by microscopic method, using the formalin-ether concentration technique. The use of *Toxocara* larval stage excretory secretory antigens in the form of serological tests seems to be a convenient method to detect anti-*Toxocara* antibodies in dogs' sera in epidemiological studies, which provides a proper picture of the prevalence of this parasite.

The present study was conducted, using an in-house indirect ELISA system with *Toxocara* larval stage excretory-secretory antigens, to determine the prevalence of anti-*Toxocara* antibodies in dogs in Fars province, southern Iran.

Materials and Methods

Study area

This descriptive cross-sectional study was conducted in 3 rural areas (Sar Mashhad, Hossein Abad, and Tolesaman) in Kazeroon Township in Fars Province, southern Iran. The residents of the mentioned area are often engaged in agriculture and animal husbandry. The region has hot summers and mild winters and is located at the border of Fars and Bushehr provinces (9).

Sampling

Serum samples were prepared from both stray and domestic dogs in our previous study

in 2018, preserved in -70 °C freezer (10). The present study was conducted in 2022 on these samples. Serum samples were analyzed by indirect ELISA for the presence of anti-*Toxocara* antibodies. Demographic information including age (estimated by teeth examination) and gender of dogs was recorded. In the studied area, owned dogs, like stray dogs, are kept outdoors. They roam freely in the environment.

Detection of anti-Toxocara antibodies

An indirect-ELISA system based on excretory/secretory antigens (TES) of *Toxocara* larvae was utilized to detect anti-*Toxocara* antibodies in the dog's sera. TES was prepared as previously reported (11). In brief, *T. canis* eggs were extracted from the female uteri worms. They were incubated at 25 °C for 30 days in a 2.5% formalin/ringer solution to become embryonated. The second-stage larvae were cultivated in RPMI medium. The culture supernatant, containing the TES antigens, was concentrated; its protein content was measured and stored at −20 °C until use. Flat-bottom microplates (96-well, Corning, USA) were coated with *Toxocara* antigen (5 µg/ mL) and incubated overnight at 4 °C. The plate was washed five times by washing buffer (phosphate-buffered Saline-Tween (PBST): 0.05% Tween 20 in PBS) via an automated ELISA plate washer. Skimmed milk (5% in PBST) was used to block the wells; after that, sera (1/100 dilution in PBST) were added (100 µL/well) to the plate and incubated at room temperature for 1 hour. The washing procedure was repeated as before. Then, 100 µL (1/4000 dilution in PBST) of peroxidase-labeled anti-canine IgG (Sigma-Aldrich, St Louis, MO, USA, anti-dog IgG peroxidase antibody produced in rabbit) was added to each well and incubated for 1 hour at room temperature. Following the washing step, as before. O-phenylenediamine (OPD) solution (0.4 mg/mL OPD, 0.3% H_2O_2) was added, and the optical density (OD) was calculated at 490 nm using a microplate ELISA reader. In each run of ELISA, one *Toxocara* positive control serum and 5 negative sera samples, as negative control, and two wells, one without serum and the other without antigen, were considered as controls. The *Toxocara* positive control sample was obtained from a dog infected with *Toxocara* from Shiraz Veterinary Faculty.

The cut-off point was determined by the mean OD of negative samples plus two standard deviations (SD). Finally, the borderline and the positive samples were retested to confirm the results.

Statistical Analyses

SPSS v.22 (IBM Corp., Armonk, NY, USA) was used for data analysis. The chi-squared test was used to assess the associations between toxocariasis seropositivity and ordinal and nominal variables. *P*-value was considered significant at the level of <0.05.

Results

Anti-*Toxocara* antibodies were detected in sera of 32 of 60 dogs corresponding to a seroprevalence rate of 53.3%. The validity of the results was confirmed by retesting the samples. The age range of the dogs was between 1 to 7 years with a mean age of 2.97 (±1.4 years). Although the seropositivity rate of toxocariasis was higher in older dogs, there was no significant association between the seropositivity to toxocariasis and dogs age. Out of 32 seropositive cases, 21 (60%) were male, and 11 (44%) were female. The difference between dogs' gender and seropositivity to *Toxocara* was not statistically significant (*P*>0.05). Table 1 demonstrates the demographic features of the studied dogs and relative seropositivity to toxocariasis.

Table 1: Characteristics of evaluated dogs and relative seropositivity to toxocariasis in a rural area of Fars Province, southern Iran

Variable	*Number*	*No. Positive for anti-Toxocara antibodies (%)*	*P-value*
Age*			
≤ 3 years	25	12 (48)	0.457
> 3 years	9	7(77.7)	
Gender			
Male	35	21(60)	0.288
Female	25	11(44)	
Area			0.167
Sar Mashhad	23	9(39.9)	
Hossienabad	13	7(53.8)	
Tolesaman	24	16(66.7)	

*Data related to age were not available in 26 cases

Discussion

The first step in the prevention and control of zoonotic parasitic diseases is to know the epidemiology of the disease, especially in the definitive host. Several studies have been conducted on the prevalence of animal toxocariasis in Iran, however, there are still many rural and remote areas where the prevalence of this disease is unknown. The seroprevalence of canine toxocariasis in our study region was reported to be 53.3%. The high prevalence of toxocariasis in the present study and most of the similar studies conducted in Iran indicated the undeniable role of domestic dogs, like stray dogs, as the source of *Toxocara* infection (12-14). The lack of appropriate control measures for dogs and the suitable environmental conditions for the survival and embryonation of *Toxocara* eggs are two main reasons for the relatively high prevalence of the infection in the epidemiological studies of canine toxocariasis (15, 16). Prenatal and lactational transmission of *T. canis*, lack of knowledge of their owners about infection, and the contamination of dog food with soil or the consumption of infected paratenic hosts can be attributed to the high level of *Toxocara* infection in dogs in rural areas (17, 18).

In a previous study, the prevalence of *T. cati* in 30 autopsied stray cats was reported to be 26.7% in Fars Province, where the current study has been done (19). In another study, the prevalence of *T. cati* in stray cats of Shiraz was 42.6% (20). On the other hand, the considerable soil contamination with *Toxocara* eggs in Fars province justifies the high prevalence of animal toxocariasis in our study area (7, 21).

The higher prevalence in male (60%) compared to female (44%) dogs in our study was consistent with previous studies (13, 14, 22). Male hormonal factors play as a risk factor for intestinal parasites, including *Toxocara* infection in dogs (23).

Although the rate of *Toxocara* infection in older dogs was higher, however this rate was not significantly different based on dogs age. The higher seroprevalence of toxocariasis in older dogs in our study can be explained by two factors: 1) longer exposure of older dogs to the sources of infection, 2) infection of dogs at a younger age and the survival of anti-*Toxocara* antibodies at older ages due to the presence of parasite larvae in the tissues of infected dogs.

The rate of *T. canis* infection reported in this study was higher than those reported in some other areas of Iran including Khorasan (11.5%) (24), Ilam (36.61%) (25), West Azerbaijan (31.33%), (26), Kerman (10%), (27), Hamadan (6.27%), (28) and Zanjan (1.78%) (29). On the other hand, studies conducted by Daryani et al. in Mazandaran and Meshgi et al. in Tehran reported higher prevalence of *Toxocara* infection, 60% and 53.8%, respectively, in dogs in Iran (30, 31). Most of the studies conducted in other countries reported a lower prevalence of *Toxocara* infection in dogs (32-37). However, two studies carried out in Denmark and Greece reported a higher prevalence, 79% and 66.7%, respectively for *Toxocara* infection in dogs (38, 39).

Differences in the rate of *Toxocara* prevalence in the above-mentioned studies can be due to the variation in sample size, the breed of the investigated dogs and the applied diagnostic methods (22).

Although the coprological method is considered the gold standard in the diagnosis of *T. canis* infection through the detection of *Toxocara* eggs in the stool sample, yet this method is unsuitable for the epidemiological studies due to the inability of the method to identify immature parasites when the egg excretion in not started, and also the expert's role in identifying the parasite eggs, because of improper sampling or fecal debris. In addition, this method cannot detect previous infection in dogs when the adult worm is no longer in the intestine.

Serological methods such as ELISA, which is routinely used in the diagnosis of human toxocariasis, can be considered a promising alternative method for the diagnosis of animal toxocariasis (40, 41). In the study by Meshgi et al., two diagnostic methods including sedimentation and Dot-ELISA were assessed for the detection of feline toxocariasis, and the prevalence of infection was higher (53.8% in comparison with 40%) by the serological method (31). The high prevalence of *Toxocara* infection in dogs in our study can also be due to the use of a serological method that identifies current as well as previous infections in dogs or, in other words, identifies the exposure of dogs with this parasite.

It is worth to mention that one of the main limitations of the present study was the small number of examined samples. In addition, missing the age of some dogs can be considered as another limitation of this study.

Conclusion

The high prevalence of *Toxocara* infection in dogs in the current study confirms that infected dogs are an important source of *Toxocara* infection for their owners and people who are in close contact with these animals, especially children. It is necessary to carry out appropriate control measures and screening programs on rural domestic dogs to stop the transmission cycle of this infection in such areas.

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Conflicts of interest

None to declare.

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