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A seroepidemiological survey of *Toxoplasma gondii* infection in referred dogs to Veterinary Hospital of Ahvaz, IranFatemeh Zarra-Nezhad^{a,*}, Mahdi P. Borujeni^a, Bahman Mosallanejad^b, Hossein Hamidinejat^c^a Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran^b Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran^c Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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ABSTRACT

Toxoplasma gondii is an intracellular protozoan parasite, which is the cause of toxoplasmosis and can infect a wide variety of warm-blooded animals, including dogs and humans. The present study evaluated the seroprevalence of *T. gondii* infection in pet dogs in Ahvaz, southwest city of Iran, and investigated the related possible risk factors. A total of 180 serum samples were collected from dogs referred to Veterinary Hospital of Ahvaz. The samples were then tested by indirect enzyme-linked immunosorbent assay. The prevalence of *T. gondii* antibodies were 46.67%. Logistic regression and chi square tests were used for evaluating of risk factors. The positivity increased statistically significantly with dog's gender (56% females and 39% males, $P = 0.001$), age (18% in <2 years old, 96% in ≥ 4 years old, $P = 0.001$) and place of living (47% outdoor dogs 38% house hold dogs, $P = 0.025$). However, no statistically significant association was found with dogs' breed, deworming, food ingestion or contact with cats. Overall, the results showed a relatively high seroprevalence of *T. gondii* infection in dogs in southwest Iran (Ahvaz) and proved association of *T. gondii* prevalence rates with the dog's age, gender and place of living.

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1. Introduction

Apicomplexan parasite *Toxoplasma gondii* (*T. gondii*) is a common infection that can infect a large number of warm-blooded animals [1]. The only definitive host known for *T. gondii* are Felids [2], which are the most important source of infection, therefore other animals as well as felids are considered as intermediate hosts. Generalized toxoplasmosis may occur in dogs under one year and is characterized by vomiting, fever, diarrhea, tonsillitis, icterus, and dyspnea [3]. In some cases, clinical toxoplasmosis in dogs might be misdiagnosed as canine distemper virus infection because it can infect and cause disease in immunosuppressed patients [4].

The source of *Toxoplasma gondii* infection is obtained by consumption of raw meat containing tissue cysts or through water and food contaminated with *T. gondii* oocysts [5]. Dogs can also play a role in the mechanical transmission of *T. gondii* oocyte to human through swallowing the infected feces of cats [6,7]. Accordingly, the seroprevalence of *T. gondii* infection in dogs may reveal the level of parasite contamination in their environment [8]. There-

fore, dogs are used as sentinel animals for *T. gondii* infection because of their close contact with humans [8,9].

T. gondii infection in dogs is spread worldwide, with seroprevalence levels going from 20% to 91% in different states [10–14]. In the present study, the serum samples were tested with indirect enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. ELISA with adequate sensitivity and specificity offers valuable data about *T. gondii* infection and many other infectious diseases, which has been used in previous studies in Iran and other countries. The aim of the study was to detect *T. gondii* antibodies and analyze risk factors of infection in dogs from southwest of Iran.

2. Materials and methods

2.1. Study samples

This study was performed in city of Ahvaz, southwestern of Iran, situated at an altitude of 12 m above sea level where the typical weather is warm and humid. A total number of 180 serum samples were randomly collected from owned dogs of various ages, breeds and either gender during 2015–2016. Dogs were brought to Veterinary Hospital of Shahid Chamran University, a

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* Corresponding author.

E-mail address: Fzaranezhad@gmail.com (F. Zarra-Nezhad).

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facility which provides a full range of services from routine check-ups and vaccinations to emergency care and surgical procedures. The referred dogs were apparently in good health and were referred for vaccination purposes. From 180 samples, 78 (43%) were females and 102 (56%) were males. Age was determined by tooth replacement in dogs. The dogs were divided into three groups: less than 2 years old; 2–4 years old and 4 and over 4 years old. Blood samples were collected from cephalic vein and then centrifuged at 2500g (gravity) for 8 min. Subsequently serum was separated and stored at -20°C until ELISA examination. Information about dog's gender, age, and breed were documented in an enquiry form.

2.2. Serological test

The ELISA test was done according to the producer's instructions (ID Vet innovative diagnostics, rue Louis Pasteur, Grabels, France) and the optical density of the samples was measured at 450 nm [15]. For each sample, scotopic photopic (S/P) (%) was calculated:

$$S/P\% = \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{\text{PC}} - OD_{\text{NC}}} \times 100 \quad (1)$$

Samples with $S/P \geq 70\%$ were considered positive.

2.3. Statistical analysis

All the analyses were carried out using SPSS (Version 16.0; SPSS Inc., Chicago, USA). The association between genders, age, breed, food, history of deworming, living places, type of use and contact with cats were analyzed by kolmogorov-smirnov test, logistic regression and chi-square test. P -value of ≤ 0.05 indicated statistically significant differences. All Numbers and categories are mentioned in Table 1.

3. Results

Among the 180 sera, 84 samples (46.67%, 95% CI: 39.37–53.97%) had antibodies against *T. gondii* and were positive. The seroprevalence of *T. gondii* infection in females was higher (56%) than males (39%) ($P = 0.001$). The seroprevalence of *T. gondii* infection varied in different age groups, ranging from 18% in <2 years old, to 96% in ≥ 4 years old, which increased with increasing of age ($P = 0.001$). Frequency of infection in dogs that had access to outdoors such as parks was higher (47%) compared to those did not access (38%). Overall the positivity increased statistically significantly with dogs' gender, age and place of living. *T. gondii* in pet dogs (43%) had a lower seroprevalence than guard dogs (53%, $P > 0.05$). No statistically significant differences were found in seroprevalence between different breeds ($P > 0.05$). Infection rate in dogs fed with cooked meals was 1.66% (95% CI: 0.63–4.34) ($P \geq 0.05$) compared to those fed with undercooked meals with no statistically significant difference. Dogs with history of deworming had a lower seroprevalence (45%) compared to dogs that had not been dewormed (63%). The results are summarized in Tables 1 and 2.

4. Discussion

In the present study, seroprevalence of *T. gondii* infection in pet dogs in city of Ahvaz, was 46.67%. Previous surveys reported varying seroprevalence of *T. gondii* infection in pet dogs in Iran: 48% in Northwest of Iran [16], 31% in Shiraz [17], 26% in Charamahalebakhtiari, Esfahan, and Khuzestan province [18], and 22% in Tehran [4]. Seroprevalence of *T. gondii* infection in dogs was reported in several locations other than Iran. For example, seroprevalence of *T. gondii* infection in dogs in Brazil was 25–62% [19,20] and in Czech Republic, 25–50% [21]. Furthermore, infection rate was 51% in Turkey [22], 33% in Sweden [23], 26% in Austria [24], and 12% in Spain [25].

Moreover, the results of present study revealed that seroprevalence rates were highly associated with gender, age and place of living. However, no statistically significant association was found

Table 1
Associations between the variables and the presence of anti-*Toxoplasma gondii* antibodies in dogs from southwest Iran.

Category	Groups	Prevalence (%)	Odds Ratio	95% CI for OD	P-Value
Age	<2 years	18 (16/88)	–	–	
	2–4 years	62 (38/61)	4.33	2.86–6.56	<0.001
	≥ 4 years	97 (30/31)	–	–	
Gender	Female	56 (44/78)	2.01	1.1–3.65	<0.05
	Male	39 (40/102)	–	–	–
Breed [*]	Large	46 (61/131)	–	–	
	Small	46 (23/49)	1.02	0.53–1.96	>0.05
Access to parks	Yes	47 (79/167)	1.44	0.45–4.57	>0.05
	No	38 (5/13)	–	–	–
Contact with cats	Yes	75% (9/12)	3.72	0.97–14.23	>0.05
	No	44 (75/168)	–	–	–
Type of use	Pet	43 (51/118)	–	–	–
	guard	53 (33/62)	1.5	0.81–2.77	>0.05
Food	Cooked	45 (73/161)	1.66	0.63–4.34	>0.05
	Raw	57 (11/9)	–	–	–
Living place [*]	Indoors	37 (18/48)	–	–	–
	Outdoors	50 (66/132)	1.67	0.85–3.25	>0.05
Deworming	Yes	45 (77/169)	–	–	–
	No	63 (7/11)	2.09	0.59–7.41	>0.05

Note 1: ^{*}Small dog breeds such as Chihuahua and terriers that are more suited for life in apartments and large dog breeds such as Labrador Retrievers and German Shepherds are more athletic breeds which need a larger household. Because some variables were not statically significant, those variables with higher insignificance had been removed. The final result is shown in Table 2.

Note 2: ^{*}In living place category Indoor reflects the dogs that were kept inside the house and outdoor reflects dogs that were kept outside the house and in the yard.

Table 2
Odds ratio in multivariable logistic regression.

Category	Groups	Odds Ratio	95% CI for OD	P-Value
Age	<2 years	–	–	–
	2–4 years	5.4	3.31–8.23	0.001*
	≥4 years	–	–	–
Gender	Female	4.75	1.84–12.25	–
	Male	–	–	0.001*
Living place	Indoors	–	–	0.025*
	Outdoors	3.34	1.17–9.59	–

Note: * indicates significance at 0.95%.

with breed, deworming, ingestion of raw meat or contact with cats. The result showed that seroprevalence was higher in female dogs (56%) than males (39%), which is in accordance with findings of Hosseini et al. [4] and Shadfar et al. [26] in Iran. Higher risk of infection in females can be due to lower immune system in female dogs during and after pregnancies and lactation, yet other findings in Taiwan [27] and Iran [18,28] reported no significant association of the infections with dog's gender. Results of the present study were in line with findings from Brazil [11] and Iran [18,28,29], indicating that the chance of having *T. gondii* antibodies increases with age and has been ascribed to higher possibility for exposure to these protozoan parasites over time, rising the exposure in older dogs [18]. Findings of this study concerning dog's living places were also consistent with previous findings suggesting that infection rate is remarkably higher in outdoor dogs compared to indoor dogs [4,18]. One explanation is that, higher seroprevalence of *T. gondii* in dogs that are kept outdoors may be related to their accessibility to risk factors such as infected intermediate hosts, uncooked meat and also exposure to soil, water or food containing sporulated *T. gondii* oocysts [18,30,31]. Warm and humid climatic conditions in city of Ahvaz, might increase the risk of survival and spread of the oocysts, and therefore cause high *T. gondii* infection in this region. The present result concerning ingestion of raw meat, was in line with previous findings, suggesting that feeding dogs raw meat can be considered a risk factor for *T. gondii* infection but was not statistically significant [26,32]. Finally, no statistically significant association was found between the infection rate and breed, deworming, or contact with cats, which is in line with some previous studies [9,4,8].

Consequently, the results of present study revealed that *T. gondii* infection may cause a major threat toward safety and public health in city of Ahvaz. However, more research is needed in order to find the causes of environmental infection with *T. gondii* and prevent it in dogs as well as humans living in this area. If individuals, especially those with immunodeficiency, are exposed to cats, dogs and soil, applying the hygienic principles is necessary to prevent them from being infected. Though, further research in different regions of Iran, is required to investigate the total epidemiological status of *T. gondii* infection in dog's population.

5. Conclusions

T. gondii infection among pet dogs in Ahvaz city from southwest, Iran is very high (46.67%). The seroprevalence rates are associated with dog's gender, age and place of living. Moreover *T. gondii* infection can be an important risk factor for public health in Ahvaz. Consequently, the findings of this study may benefit future studies and extend public awareness of the epidemiology of *T. gondii* infection in dogs in Iran. Early detection and control of toxoplasmosis in cats that live in the studied areas can reduce the incidence of the infection in humans and other intermediate hosts. Further studies

are recommended to investigate the occurrence of *T. gondii* oocysts in dog's feces in association with serology and molecular diagnosis.

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Competing interests

We confirm that there are no known conflicts of interest associated with this publication.

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