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Original Article
Özgün Araştırma

Seroprevalence and risk factors for *Toxoplasma gondii* infection in shelter cats in Erzurum province of Türkiye

Başak Hanedan[1*], Cahit Babür[2], Muhammed Sertaç Eroğlu[3], Selin Sinem Sümbül[4], Ömer Alkan[5]

[1,3,4] Atatürk University Faculty of Veterinary Medicine, Department of Veterinary Internal Medicine, Erzurum, Türkiye
[2] Department of National Parasitology Reference Laboratory, Public Health General Directorate of Turkey, Ankara, Türkiye
[5] Atatürk University Faculty of Economics and Administrative Sciences, Department of Econometrics, Erzurum, Türkiye

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Abstract: This study was aimed to evaluate the seroprevalence and risk factors of the infection of *Toxoplasma gondii* in cats kept in Animal Shelter and Rehabilitation Center of Erzurum Municipality in Erzurum that collected from stray. Total 70 blood samples were collected from apparently healthy cats and diseased cats such as diarrhea, and ocular and nasal discharge. Sera samples were stored in screw-capped sterile tubes at – 20 °C until analysis. In sera samples, IgG antibodies against *T. gondii* were analyzed by Sabin-Feldman Dye test. Of 70 cats, 25 (35.7%) was negative and 45 (64.3%) positive for *T. gondii* infection. An important association was not found between young and adult, female and male, and apparently healthy and diseased cats with *T. gondii* seropositivity. In this study, *T. gondii* seroprevalence was determined at high rates in cats kept in shelter in Erzurum province of Turkey. The prevention measures against *T. gondii* infection in cats should be implemented for animal and public health concerns.

Keywords: *Toxoplasma gondii*, risk factors, seroprevalence, cat

Türkiye'nin Erzurum ilinde barınak kedilerinde *Toxoplasma gondii* enfeksiyonu için seroprevalans ve risk faktörleri

Özet: Bu çalışmada Erzurum İlinde sokaktan toplanan Erzurum Büyükşehir Belediye'sine ait Hayvan Bakımevi ve Rehabilitasyon Merkezi'nde bakılan kedilerde *Toxoplasma gondii* enfeksiyonunun seroprevalansı ve risk faktörlerinin araştırılması amaçlandı. İshal ve göz ve burun akıntısı gibi hastalıklı kediler ve görünüşte sağlıklı kedilerden toplam 70 kan örneği toplandı. Serum örnekleri vida kapaklı steril tüplerde analize kadar -20 °C'de saklandı. Serum örneklerinde *T. gondii*'ye karşı IgG antikorları Sabin-Feldman Dye testi ile analiz edildi. *T. gondii* enfeksiyonu için 70 kedinin 25'i (%35,7) negatif ve 45'i (%64,3) pozitif idi. *T. gondii* seropozitifliğiyle genç ve erişkin, dişi ve erkek ve görünüşte sağlıklı ve hasta kediler arasında önemli bir ilişki belirlenmedi. Bu çalışmada Türkiye'nin Erzurum İli'nde barınakta bakılan kedilerde *T. gondii* seroprevalansı yüksek oranlarda belirlendi. Kedilerde *T. gondii* enfeksiyonuna karşı korunma önlemlerinin hayvan ve halk sağlığı sorunları bakımından uygulanması gerekmektedir.

Anahtar kelimeler: *Toxoplasma gondii*, risk faktörleri, seroprevalans, kedi

Introduction

Toxoplasmosis is a protozoan disease occurring in animals and humans all over the world. *Toxoplasma* (*T.*) *gondii* is a coccidian parasite that can infect almost all animals. Oocysts are shed in cat feces. Oocysts are sporulated 1-5 days after they are shed with feces (Datz and Evason 2020). They can survive in the environment for months or even years. The soil contamination with oocysts indicates ongoing contamination (Awobode et al., 2020).

Toxoplasmosis is widespread throughout the world, is more common in temperate and tropical climates and less common in dry and cold climates (Datz and Evason 2020). Cats infected with *T. gondii* are likely to have lifelong tissue cysts. Therefore, the presence of serum antibodies is likely to indicate the current infection. Since the risk of exposure increases over time, the increase in seroprevalence is correlated with advancing age (Lappin 2014).

Common clinical findings are anorexia, weakness, fever, respiratory distress, diarrhea, gastritis and neurological disorders (Lappin 2014; Datz and Evason 2020). In addition, lethal toxoplasmosis occurs when the liver, lung, central nervous system and pancreatic tissues are affected (Lappin 2014).

Yazışma adresi / Correspondence: *Başak Hanedan, Atatürk University Campus Veterinary Faculty, Yakutiye, Erzurum, Türkiye*
e-mail: basak.hanedan@atauni.edu.tr

ORCID IDs of the authors: *[1]0000-0003-3873-0124 • [2]0000-0002-6524-3260 • [3]0000-0003-1061-8421 • [4]0000-0002-8680-5402 • [5]0000-0002-3814-3539*

Toxoplasmosis in humans is caused by exposure to the contaminated meat or infected cat feces (Dijurkovic-Djakovic et al., 2019), as well as by drinking contaminated water, eating fruits and vegetables contaminated with spore-forming oocysts (Dubey 2009). Especially, the feces of infected cats are an important source of contamination (Montazeri et al., 2020). After transplacental infection in humans, it poses a significant risk in the fetuses and immunocompromised patients. Most people become infected by eating spore-forming oocysts or tissue cysts or transplacentally (Lappin 2014).

T. gondii seroprevalence was reported to be 5.6%-6.7% in shelter cats in different years in Japan (Oi et al., 2015), 64.48% in stray cats in Iran (Tehrani-Sharif et al., 2015), 19.2% in shelter cats in Scotland (Bennett et al., 2011), 44.1% in cats with free-roaming and indoor in Kars, Turkey (Erkılıç et al., 2016), 41.0% in pet cats in Norway (Saevik et al., 2015), 52.8% in stray cats in Ankara, Turkey (Özkan et al., 2008), 76.4% in stray cats in Niğde (Karatepe et al., 2008), 32.11% in domestic and feral cats in South Africa (Tagwireyi et al., 2019), 14.1% in stray cats in Korea (Par et al., 2020), 86.7% in stray cats in Nigeria (Awobode et al., 2020), and 35% and %59 in domestic and feral cats globally, respectively (Montazeri et al., 2020).

An important risk factor in cats is hunting behavior and ingestion of prey. Eating raw or undercooked meat is also a common mode of transmission. Seroprevalence is higher in adult cats, in free-roaming cats, and in shelter cats (Datz and Evason 2020).

Exposure to cats and litter boxes is thought to be an important risk factor for humans (Datz and Evason 2020). Since cats are in close contact with humans, cats are potential sources of *T. gondii* (Oi et al., 2015). Toxoplasmosis is a zoonotic parasitic disease that is common in animals and humans around the world (Bilgili and Hanedan 2019). It has been reported that more than 1 billion people in the world are infected with *T. gondii* (Hoffmann et al., 2012). Therefore, in order to protect human and animal health, there is a need to know the current situation of toxoplasmosis in cats in our country and to implement ways to prevent this disease.

In Turkey, less studies on the seroprevalence of *T. gondii* in cats are carried out and one study on risk factors is observed (Yücesan et al., 2019). In this study, it was aimed to investigate the seroprevalence and risk factors of *T. gondii* infection in shelter cats in Erzurum, Turkey.

Materials and methods

Animal material

This study was funded by Atatürk University Scientific Research Projects Coordination Unit on 30.09.2021 with the code TKP-2021-9600. In addition, this study was approved by Atatürk University Animal Experiments Local Ethics Committee (27.05.2021/119). In this study, blood samples were collected from 70 cats living in Animal Shelter and Rehabilitation Center of Erzurum Municipality in Erzurum, Turkey, were centrifuged, and sera samples were stored in screw-capped sterile tubes at -20°C until analysis. In the sera samples, IgG antibodies against *T. gondii* were analyzed by Sabin-Feldman Dye test. Young or adult, gender, breed, feeding regimens, place where they were brought, and apparently healthy or diseased conditions for each animal were noted. They were crossbreed and stray cats.

Sabin-Feldman Dye test

The seropositivity of *T. gondii* in cats was analyzed by the Sabin-Feldman Dye Test (SFDT) (Sabin and Feldman 1948; Katsube et al., 1972). In the analysis, the dilutions of positive and negative controls and the test sera were performed using 0.9% saline in a 4-fold serial dilutions (1:4-1:1,024). After diluted, 25 µl was transferred to a tube and an equal volume of activator serum rich in C2, C3, C4 and properdin was added. After 48 hours, tachyzoites (creating a density of approximately 25 tachyzoites per 400x microscope field) were added to each tube. The incubation of tubes were carried out at 37°C for 50 min. Afterwards, an additional 10 min incubation at 37°C was performed in the presence of 25 µl of alkaline methylene blue (pH 11). After incubation, 20 µl of each sample was examined at a 40x objective. If more than 50% of the observed *T. gondii* tachyzoites remained unstained, dilutions were considered positive.

Statistical analysis

Frequency analyzes related to the seropositivity status of cats were calculated. Chi-square independence test was used to investigate the association between risk factors and seropositivity status.

Results

Of 70 cats, 25 (35.7%) were negative and 45 (64.3%) were positive for *T. gondii* seropositivity. The frequency and percentage distributions of cats according to *T. gondii* seropositivity titers are given in Table 1.

The associations between cats' *T. gondii* seropositivity status and risk factors were given in Table 2. Cats were classified according to age as young and adult. Of the youngs, 19 were positive, 14 were negative, and 26 of the adult cats were positive and 11 were negative. 21 of the female cats were positive, 14 were negative. 24 of the male cats were positive and 11 were negative. Of the ill cats, 20 were positive and 11 were negative, and 25 of the apparently healthy cats were positive and 14 were negative. It was determined that there was no significant relationship between *T. gondii* seropositivity and age, gender and health status.

Table 1. Frequency and percentage distributions of cats according to *T. gondii* seropositivity titers

Titer	Frequency	Percent (%)
1/16	35	50
1/64	7	10
1/256	3	4.3
Negative	25	35.7

Table 2. Results of associations between *T. gondii* seropositivity and risk factors

Variable	Category	Positive	Negative	Total	Prevalence (%)
Age	Young	19	14	33	57.6
	Adult	26	11	37	70.3
P = 0.26; df = 1; chi square value = 1.224					
Gender	Female	21	14	35	60.0
	Male	24	11	35	68.6
P = 0.45; df = 1; chi square value = 0.560					
Status of health	Apparently healthy	25	14	39	64.1
	ill	20	11	31	64.5
P = 0.97; df = 1; chi square value = 0.001					

Discussion and Conclusion

This study was first on *T. gondii* seropositivity and risk factors in shelter cats in Erzurum, Turkey. The seroprevalence of *T. gondii* in shelter cats in Erzurum, Turkey was determined to be 64.3%. The seroprevalence rate in shelter cats in this study was higher than 44.1% in cats with outdoor access in Kars city (Erkılıç et al., 2016), 52.8% in stray cats in Ankara city (Özkan et al., 2008), 42-48% in 1021 apparently healthy stray cats and 37.84% in stray cats in İzmir city (Can et al., 2014; Karakavuk et al., 2021), but was lower than 76.4% in stray cats in Nigde city (Karatepe et al., 2008).

In the studies investigating the seropositivity of *T. gondii* in cats around the world, the seroprevalences of infection have been reported to be 64.48% in stray cats in Iran (Tehrani-Sharif et al., 2015), 86.7% in stray cats in Nigeria (Awobode et al., 2020), 82% in feral cats in Qatar (Boughattas et al., 2017), 57.1% in shelter cats in Estonia (Must et al., 2015), 22.3% in stray cats in Greece (Kokkinaki et al., 2023), 20.9% in stray cats in China (Xia et al., 2022), and 14.1% in stray cats in Korea (Park et al., 2020). In addition, globally, the seroprevalence of *T. gondii* has been determined to be 4.8% to 97.4% in domestic cats (Jittapalapong et al., 2010; Al-Kappany et al., 2010).

Accordingly, *T. gondii* seropositivity is appeared to be high in stray cats in Turkey and in many countries in the world. In this study, when the positivity titers of infected cats were examined, it was determined that there were 35 cats with 1/16 titer, 7 cats with 1/64 titer and 3 cats with 1/256 titer. High-titer antibody responses do not always require cats to have been recently infected (Afonso et al., 2007).

Cat species are highly susceptible to *T. gondii* infection and a single tissue cyst bradyzoite can cause infection (Dubey 2009). Raw or undercooked food and hunting rodents and birds are the important risk factors (Saevik et al., 2015).

In this study, no significant association was found between *T. gondii* seropositivity and gender in cats consistent with the study by Boughattas et al. (2017) in stray cats in Qatar However, Saevik et al. (2015) have reported a significant association with seropositivity in male cats compared to female cats. Finding different results between infection positivity and gender in different studies may be due to the geographical region studied, study population, and

statistical methods (Jittapalapong et al., 2007; Saevik et al., 2015), as well as male cats hunting more and exposure to infection more (Afonso et al., 2007).

In this study, no significant association was found between *T. gondii* infection positivity and age consistent with the study implemented in stray cats by Matsuu et al. (2017) in Japan. However, in other studies, it has been reported that infection positivity increases significantly in adult cats (Tehrani-Sharif et al., 2015; Must et al., 2015; Bawm et al., 2020). In this study no significance between age and seropositivity might be attributed to similar exposure of *T. gondii* via ingesting oocysts and/or tissue cysts in youngs and adults living outdoors or IgG positivity of *T. gondii* in youngs until 12 weeks of age might be derived from colostral IgG transfer (Gauss et al., 2003; Kokkinaki et al., 2023) or transplacental infection (Xia et al., 2022).

It can be expected that aging, free-roaming and hunting behavior in cats would increase infection rates. Similarly, Brennan et al. (2020) have found the risk factors for *T. gondii* infection in cats as older than 1 year, eating raw meat, and hunting behaviors. Eating cystic meat containing *T. gondii* bradyzoites causes an increase in the infection rates (Salman et al., 2018).

In this study, no significant association was found between the health status of cats and *T. gondii* positivity. Consistent with this study, Saevik et al. (2015) have reported that they do not detect a significant relationship between *T. gondii* positivity and health status in cats. The rates of seropositivity prevalence in apparently healthy and diseased cats (diarrhea and/or respiratory problems) were highly increased as 64.1% and 64.5%, respectively.

As the infected cats shed *T. gondii* oocysts with feces, they cause contamination of soil and water and health problems in animals and humans (Bawm et al., 2020). Cats develop antibodies against *T. gondii* 1-2 weeks after infection (Dubey and Lappin 2012). Therefore, it is important to investigate the presence of *T. gondii* in feces, body fluids, tissue samples and antibodies in serum together in the evaluation of *T. gondii* infections in cats. Investigation of *T. gondii* oocysts in the feces of cats with high-sensitivity techniques such as PCR, colorimetric loop-mediated isothermal magnification analysis (Karakavuk et al., 2022) and making the necessary treatments of positive cases are important in protecting other vertebrate animals and humans against *T. gondii* infections.

In this study, *T. gondii* seroprevalence was determined at high levels in cats living in shelter in Erzurum, Turkey. This suggests that the distribution of *T. gondii* oocysts is prevalent in the environment. The prevention measures against *T. gondii* infection in cats should be implemented.

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Conflict of Interest: This study authors have no conflicts of interest.

Ethics Committee Approval: This study was approved by Atatürk University Animal Experiments Local Ethics Committee (27.05.2021/119).

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