Bozok Veterinary Sciences

Bozok Vet Sci
(2021) 2, (2):29-33

Araştırma Makalesi / Research Article

Seroprevalence of Canine Toxoplasmosis by Sabin Feldman Dye Test in Konya Province, Turkey*

Firas ALALİ[1]✉, Ferda SEVİNÇ[2], Cahit BABÜR[3], Onur CEYLAN[2]

[1] University of Kerbala, College of Veterinary Medicine, Department of Veterinary Parasitology, Karbala/IRAQ
[2]Selcuk University, Faculty of Veterinary Medicine, Department of Veterinary Parasitology, Konya/TURKEY
[3]General Directorate of Public Health, Microbiology Reference Laboratories and Biological Products Department of National Reference Laboratory of Parasitology, Ankara/TURKEY

◆ **Geliş Tarihi/Received:** 20.11.2021 ◆ **Kabul Tarihi/Accepted:** 13.12.2021 ◆ **Yayın Tarihi/Published:** 31.12.2021

Bu makaleye atıfta bulunmak için/To cite this article:
Alali F, Sevinç F, Babür C, Ceylan O. Seroprevalence of Canine Toxoplasmosis by Sabin Feldman Dye Test in Konya Province, Turkey. Bozok Vet Sci (2021) 2, (2):29-33.

Abstract: *Toxoplasma gondii (T. gondii)* is a protozoan parasite that lives within cells and is the cause of toxoplasmosis in dogs and all animals. The purpose of this research was to determine the seroprevalence of *T. gondii* infection in stray dogs in the Konya province of Turkey. Between July 2017 and July 2018, 334 plasma samples were taken from dogs of both genders, aged 0-1 and 1-3 years. The samples were tested for *T. gondii*-specific antibodies using the Sabin-Feldman Dye Test (SFDT). *T. gondii* seroprevalence was 98.5%, with infection rates of 99.2% in males and 98.1% in females, respectively. Positive cases were 14 (100%) in 0-1 year-old animals and 315 (98.4%) in the 1-3 year age group, with no significant difference between the age groups (P>0.05). Gender differences were not statistically significant (P> 0.05). Seropositivity was detected in 98.3% (60/61) of the animals having clinical symptoms. The number of seropositive cases in new and old entrance dogs was 329 (98.5%), 42/44 (95.4%) in new entry dogs and 287/290 (99%) in old entry dogs. There was no significant difference in the elderly entry dogs (P>0.05). In the current investigation, 61 animals were examined for clinical signs, such as paralysis and atrophy of the hind-limbs, nasal discharge, skin lesions, tick infestations, vomiting, diarrhea, nervous system problems, and emaciation.

Keywords: Dog, Toxoplasmosis, Clinical signs, Turkey

Türkiye'nin Konya İli Köpeklerinde Sabin Feldman Boya Testi ile Toxoplasmosis Seroprevalansı

Özet: *Toxoplasma gondii*, köpeklerde ve tüm memelilerde toksoplazmozise neden olan hücre içi bir protozoon parazittir. Bu çalışmada Konya ilinde sokak köpeklerinde *T. gondii* enfeksiyonunun seroprevalansı araştırıldı. Temmuz 2017-Temmuz 2018 döneminde (0-1> yıl ve 1-3> yıl) her iki cinsiyetteki köpeklerden toplam 334 plazma örneği toplandı. Plazma örneklerinde Sabin-Feldman Boya testi ile *T. gondii*'ye spesifik antikorların varlığı araştırıldı. *Toxoplasma gondii*'nin seroprevalansı %98,5 bulunurken, bu değerin erkek ve dişi köpeklerde sırasıyla %99,2 ve %98,1 olduğu belirlendi. Pozitif vakalar 0-1 yaş hayvanların 14'ünde (%100), 1-3 yaş grubu hayvanların ise 315'inde (%98,4) tespit edildi ve yaş grupları arasında istatistiksel olarak anlamlı bir fark belirlenmedi (P>0.05). Cinsiyete göre seroprevalans değerleri arasında anlamlı bir fark gözlenmedi (P>0.05). Klinik belirtileri olan hayvanların %98,3'ü (60/61) seropozitif bulundu. Yeni ve eski girişli köpeklerde seropozitif hayvan sayısı 329 (%98,5) [yeni girişli hayvanlarda pozitif sayısı 42/44 (%95,4) ve eski girişli hayvanlarda 287/290 (%99)] olarak tespit edildi. Eski girişli köpeklerde seropozitiflik açısından istatistiksel olarak anlamlı bir fark tespit edilmedi (P>0.05). Çalışmadaki 61 hayvanda arka bacaklarda felç ve atrofi, burun akıntısı, deri lezyonları, kene istilası, kusma, ishal, sinir sistemi bozuklukları ve zayıflama gibi klinik belirtiler tespit edildi.

Anahtar Kelimeler: Köpek, Toxoplasmosis, Klinik bulgular, Türkiye

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite that can infect all warm-blooded vertebrates, including mammals, which may cause fatal diseases such as abortion (1, 2). Toxoplasmosis is a highly contagious illness that affects up to 90% of all animal species (3). The primary route of transmission from animals to humans is via the consumption of meat harboring tissue cysts and oocyst-contaminated water (4). In 1910, Mello reported the first case of acute visceral toxoplasmosis in a four-month-old dog in Turin, Italy.

The parasite was discovered after histological investigation of tissues harvested from the dog's ulcerated liver, lung, spleen, and intestine (5). Numerous studies have also shown that the prevalence of *T. gondii* infection in dogs ranges between 0% and 100% in various countries, and toxoplasmosis is widespread globally (5, 6). In Turkey, toxoplasmosis was reported in a dog for the first time in

✉: firas.o@uokerbala.edu.iq
*This research was a part of PhD thesis of the Firas ALALI and this study was presented as an oral at the International Congress on Biological and Health Sciences Congress (26-28 February 2021/Afyonkarahisar).

1950 (7). *Toxoplasma gondii* infections are extensively dispersed in people and animals in Turkey (8)

Clinical signs of canine toxoplasmosis include neuromuscular, respiratory, and gastrointestinal system issues. The majorities of infected dogs are asymptomatic and have additional illnesses, such as distemper and ehrlichiosis (9). This kind of infection may cause progressive brain and spinal cord damage. Peripheral neuromuscular dysfunction, atrophy of extensor muscles, and paralysis are all symptoms of this condition (10). The pulmonary form of the disease may kill dogs within a week, but the gastrointestinal form of the disease is often characterized by vomiting and diarrhea in dogs (6). Jaundice, abdominal effusion, fever, lethargy, vomiting and diarrhea may also be present in dogs with encephalopathy. Toxoplasmosis-related liver damage in young dogs is common, especially in those who have been infected with distemper. Toxoplasmosis may be passed from mother to puppy through the placenta or via the milk of the nursing mother (11).

Puppies may die from canine toxoplasmosis. High fever, tonsillitis, shortness of breath, vomiting, diarrhea, jaundice, and cardiac damage are all symptoms of infection. Ataxia, paresis, posterior limb paralysis, and hind limb paralysis are some of the other neurological signs (12). Neurological toxoplasmosis and neurological neosporosis have comparable clinical symptoms (13).

Among the serological diagnostic methods, SFDT is one of the most commonly applied methods. SFDT is one of the most frequently used serological diagnostic procedures. The SFDT seems to be the gold standard for identifying anti-*T. gondii* specific antibodies. (14,15). This study was carried out to detect *T. gondii* infections using the SFDT in stray dogs in dog shelters affiliated to Konya Metropolitan Municipality, Veterinary Department.

2. Materials and Methods

2.1. Study area

In July 2017, blood samples were obtained from all canines in Konya Metropolitan Municipality/Selcuklu region/Konya. Clinical evaluations of all canines of various genders residing in the animal shelter have been reported. All procedures were carried out in accordance with the ethical guidelines of the Experimental Animals Production and Research Center Ethics Committee of Veterinary Faculty of Selcuk University (Decision number: SUVDAMEK-2017/46).

2.2. Sample collection

Among 334 animals divided in male (124) and female (210) with different number, ages group 0-1 was (14), 1-3 group was (320), while number of clinical signs was (61) harbor two groups, new was (44) and old entry dogs was (290). Briefly, approximately 5 ml of blood was drawn from the ramus dorsal of *Vena cephalica* or *Vena saphanea parva* and placed in anticoagulant tubes. Blood was centrifuged for 15 min at 2500 rpm; plasma was collected and kept at -20°C until testing. Plasma samples were examined by SFDT for the presence of particular *T. gondii* antibodies at the Public Health Agency's *Toxoplasma* Laboratory in Ankara, Turkey's capital city.

2.3. Sabin-Feldman dye test

SFDT was performed using plasma samples of live tachyzoites and methylene-blue dye. Positive and negative controls, as well as test sera, were serially diluted fourfold with saline (1/16; 1/64; 1/256; and 1/1024). Twenty five of each dilution was transferred to a tube, and an equivalent amount of activator sera was added. The activator sera are seronegative for *T. gondii* and are high in C2, C3, C4, Mg2, and properdin. A 48-hour passage of the *T. gondii* Rh strain was obtained from the peritoneal fluid of 3-4 week old Swiss albino mice. The tubes were then incubated at 37°C for 50 min. After that, 25 µl of alkaline methylene blue (pH 11) was incubated for 10 minutes at 37°C. Following incubation, a total of 20 µl of each sample was analyzed with a 40 objective. Positive dilutions were those in which at least half of the *T. gondii* tachyzoites remained unstained. A positive antibody titer of 1:16 or greater was considered (16).

2.4. Statistical analyses

The chi-square test was used to evaluate whether there was a correlation between infection and age, gender, clinical symptoms, and new and old entrance dogs. When the probability(P)value was less than 0.05, the differences were considered statistically significant. SPSS (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical program was used to analyze all data. origin or publisher of SPSS Inc. in Chicago USA.

3. Results

The SFDT demonstrated that 98.5% of the 334 dogs evaluated were positive for anti-*T. gondii* antibodies, as shown in Table 1. A total of 99.2% (123/124) of males and 98.1% (206/210)of females were found to be positive in this investigation. The dye test was performed at 1/16, 1/64, 1/256, and 1/1024 dilutions. Seropositivity was recorded in animals, 168 (61 males, 107 females) at 1/16 dilution, 115 (43 males, 72 females) at dilution 1/64, 35 (16 male and 19 female) at 1/256 dilution, and 11 (3 males, 8 females) at 1/1024 dilutions. Table 2 shows the infection status of dogs by age. In the 0-1 age group, the SFDT indicated seropositivity in all 14/14 (100%) canines. Seven dogs were infected at a 1/16 dilution, six at a 1/64 dilution, and one at a 1/1024 dilution. In the 1-3 age group, 315 (98.4%) of 320

canines were seropositive. At dilutions of 1/16, 1/64, 1/256, and 1/1024, the number of infected dogs was 161, 109, 35, and 10 dogs, respectively.

Table 1: Distribution of infection according to genders

Gender	1/16	1/64	1/256	1/1024	n/N	%	P
Male	61	43	16	3	123/124	99.2	
Female	107	72	19	8	206/210	98.1	
Total	168	115	35	11	329/334	98.5	P>0.05

N: Total samples, **n**: Positive samples

Various clinical signs are shown in the animals investigated, including hind limb paralysis and atrophy, runny nose, skin lesions, tick infestations, vomiting, diarrhea, nervous system problems, and weakness. Table 3 demonstrates that 60 (98.3%) of the 61 animals exhibiting clinical signs tested positive for antibodies.

Table 2: Distribution of infection according to ages

Ages	1/16	1/64	1/256	1/1024	n/N	%	P
0-1	7	6	0	1	14/14	100	
1-3	161	109	35	10	315/320	98.4	
Total	168	115	35	11	329/334	98.5	P>0.05

N: Total samples, **n**: Positive samples

The proportion of infected animals with clinical signs, such as hind-limb paralysis and atrophy, skin lesions, tick infestation, hind-limb paralysis, atrophy, and skin lesions reached 100%. The percentage of animals having nasal secretions was 96.8%. At a 1/16 dilution, 15 positive animals had nasal discharge, 10 positive animals had skin lesions, 4 positive animals had tick infestations, 1 positive animal had hind-limb paralysis and atrophy, and 1 positive animal had hind-limb paralysis, atrophy, and skin lesions. The second dilution was observed in 22 animals at 1/64, and nasal discharge in 13 dogs, skin lesions in five dogs, and tick infestations in four dogs were observed. At 1/256 dilution, three dogs were tested positive, two with nasal discharge and one with skin lesions. Four dogs were detected at a dilution of 1/1024, one with nasal discharges, two with skin lesions, and one with tick infestations.

Table 3: The relationship of the infection with clinical symptoms

Clinical symptoms	1/16	1/64	1/256	1/1024	n/N	%	P
A	1	0	0	0	1/1	100	
B	15	13	2	1	31/32	96.8	
C	10	5	1	2	18/18	100	
D	4	4	0	1	9/9	100	
E	1	0	0	0	1/1	100	
Total	31	22	3	4	60/61	98.3	P>0.05

N: Total samples, **n**: Positive samples, **A**: Hindlimbs paralysis and atrophy; **B**: Nasal discharges;

C: Skin lesions; **D**: Tick infestations; **E**: Hindlimbs paralysis and atrophy and skin lesions

Seropositivity is revealed in Table 4 for new and old entrance dogs. Seropositivity in the new entry dogs was 95.4%, while it was 99% in the old entry dogs. From 44 animals at plasma dilutions of the most recent entries, there were 27 in 1/16, 12 in 1/64, 3 in 1/256, and 141, 103, 32, and 11 animals at 1/16, 1/64, 1/256, and 1/1024, respectively, from the 290 dogs that had previously been to the shelter.

Table 4: The relationship of the infection with new and old entry dogs

Clinical symptoms	1/16	1/64	1/256	1/1024	n/N	%	P
New entries	27	12	3	0	42/44	95.4	
Old entries	141	103	32	11	287/290	99	
Total	168	115	35	11	329/334	98.5	P>0.05

N: Total samples, **n**: Positive samples

4. Discussion and Conclusion

Toxoplasma gondii is a protozoan parasite that may be found in all regions of the globe and has a variety of hosts and infective stages (2). Canine toxoplasmosis has been reported in dogs under the age of one year and uncommon primary illness with clinical signs (5, 11). Dogs may be used to evaluate environmental pollution as an indicator for risk variables since they are exposed to the same infection risk as people and other animals (27). The serologic status as alternative assay for *T. gondii* in free-living animals, such as stray or free-living dogs, as an indicator, can be used to evaluate environmental contamination indirectly, as they are exposed to the same risk of infection as humans and other animals (27).

The SFDT was used to examine plasma dogs in order to investigate specific anti-*T. gondii* antibodies. The study included 334 canines and was done by the Veterinary Branch Directorate of the Konya Metropolitan Municipality. Throughout the experiment, the distribution of the infection was shown based on a number of parameters (genders, age, presence of clinical signs, new and old entries dogs) were all documented at the Shelter centre. Seropositivity was mostly found at 1/16 titer. Most results in the world meet the definition of positivity with a titer of 1/16. Dye testing is extremely specific, and its use as a reference method in all labs should be encouraged (14).

The SFDT was found to be positive in 98.5% of the dogs. This result was found to be consistent with the previous investigations findings (16-20). These findings may be attributed to increased impacts on environmental contamination in this location. Various variables, such as antibody type, sample size, and dog category, may influence SFDT results (stray, outdoor, indoor, and domesticate). Altay et al. (20) found it in 120 canine sera from Sivas province, 60 of which were stray dogs. Antibodies were found in 115 (95.8%) of the 120 serum samples. *T. gondii* seroprevalence in male dogs was 95.6%, in female dogs it was 96.2%, in the 0-2 age group it was 93.9%, in the 3-5 age group it was 95.4%, and in the over 6 age group it was 100% (20). Dogan et al. (21) discovered canine toxoplasmosis in 185 street dogs in Eskisehir Province. SFDT detected *T. gondii* seropositivity in 107 of 185 dogs (1/16 and up); no statistically significant differences were found in sex, age groups, or dog strains.

According to (Table 1), males had a 99.2% prevalence rate, while females had a 98.1% prevalence rate. There was no statistically significant difference in infection rates between males and females. It was found to be consistent with previous findings (20, 21).These explain both of genders expose to agent at the same time. The infection rate was 100% in the 0-1 age group and 98.4 % in the 1-3 age group. The findings were also noticed to be consistent with previous investigations (20, 21), (Table.2). Additionally, this location had a high rate of canine toxoplasmosis, which increased with age. The antibody titer has no impact on the severity of symptoms (6, 23).

Clinical signs of respiratory, neuromuscular, and gastrointestinal disease can be seen in the majority of dogs (6). Cerebral neosporosis is connected to the majority of toxoplasmosis symptoms in older dogs. Despite the distinctions in these diseases, the symptoms are similar (5).

In Brazil a seven-year-old female spayed Schnauzer was presented with cutaneous ulcerated nodular lesions. Biopsy showed intralesional bradyzoites cysts and tachyzoites. PCR analysis was positive for *T. gondii* (23).

In the current study, paralysis and atrophy of the hind legs, skin lesions, tick infestation, runny nose, vomiting, diarrhea, and nervous system abnormalities were recorded in 60 (98.3%) of the 61 dogs. Seropositivity was 100% in diseased animals with hind-limb paralysis and atrophy, skin lesions, and tick infestation, and 96.8% in dogs with nasal discharge. This outcome corroborated previous findings (6, 22, 23) (Table 3). In this study, the presence of a shared water source, stray cats, birds, and rodents (rats) living near stray dogs was thought to be the main reason for the risk of infection.

T. gondii is common in urban and peri-urban areas, implying that the risk factors identified for dogs were being a mixed-breed animal and living entirely outside, indicating that dogs and cats should be considered a potential risk factor for human populations (24).

The infection was found in 95.4% of newly entered dogs (42/44) and 99% of old entry dogs (287/290). This result is in line with previous investigations' findings (25, 26) (Table 4). In different studies, the rates of *T. gondii* infection have been found to vary. Dog habitats, age, and nutrition changes, close contact with the end host or reservoir hosts involved in the transfer, the absence or presence of other illnesses, immune system status, and diagnostic testing discrepancies are all factors that contribute to this condition.

In conclusion, the prevalence of canine toxoplasmosis was shown to be high. The prevalence of *T. gondii* in food, soil, and water is usually unknown, and further research is needed. Furthermore, stray dogs in Turkey are more vulnerable to infection from a variety of sources, including meat and water, and contaminated foods may pose a public health risk. Oocysts ingested via food, soil, or water is the primary risk factors. Therefore, various measures should be taken in this area to reduce the risk of toxoplasmosis.

Acknowledgement

This research was a part of PhD thesis of the Firas ALALI/Konya-2019, under the project number/17102030,

supported by Selcuk University Scientific Research Projects Coordination (BAP) and Iraqi Ministry of Higher Education and Scientific Research (MOHESR).

References

1. Ergene O, Celebi B, Kucukaslan I. Seroprevalance of canine brucellosis and toxoplasmosis in female and male dogs and relationship to various factors as parity, abortion and pyometra. Indian Journal of Animal Research. 2019; 53:954-958. doi : 10.18805/ijar.B-707.
2. Dubey JP, Murata FH, Cerqueira-Cézar CK, Kwok OC, Su C. Epidemiologic significance of *Toxoplasma gondii* infections in turkeys, ducks, ratites and other wild birds: 2009–2020. Parasitology. 2021;148: 1-30. doi:10.1017/S0031182020001961.
3. Aciöz M, Bozkaya F, Babür C. Seroprevalence of *Toxoplasma gondii* antibodies using Sabin-Feldman dye test among equines in Isparta province, Turkey. Parasitologists United Journal 2021;14:146-150. doi: 10.21608/PUJ.2021.69445.1112 .
4. Halonen SK, Weiss LM. Toxoplasmosis. Garcia HH, Tanowitz HB, Del brutto OH. In: Neuroparasitology and Tropical Neurology. British library. Radarweg, Netherland.Handb Clin Neurol 2013; 114: 125–145.
5. Dubey JP. *Toxoplasma gondii* infections in chickens (Gallus domesticus): prevalence, clinical disease, diagnosis and public health significance. Zoonoses and Public Health 2010; 57: 60-73. doi:10.1111/j.1863-2378.2009.01274.x.
6. Dubey JP, Beatttie CP. Toxoplasmosis of animals and man. United states: Boca Raton: CRC Press Inc. 1988; p.1-220.
7. Akçay S, Pamukçu M, Baran S. Bir köpekte ilk toxoplasmose observasyonu. Vet Hek Der Derg 1950;20: 245-254.
8. Kolören Z, Dubey JP. A review of toxoplasmosis in humans and animals in Turkey. Parasitology. 2020 Jan;147:12-28. doi:10.1017/S0031182019001318.
9. Pimenta AL, Piza ET, Cardoso RB, Dubey JP. Visceral toxoplasmosis in dogs from Brazil. Veterinary Parasitology1993;45:323-326.doi:10.1016/0304-4017(93)90086-3.
10. Silva NM, Lourenco EV, Silva DA, Mineo JR. Optimisation of cut-off titres in *Toxoplasma gondii* specific ELISA and IFAT in dog sera using immunoreactivity to SAG-1 antigen as a molecular marker of infection. Veterinary Journal 2002; 163:94-98. doi: 10.1053/tvjl.2001.0629.
11. Dubey JP. Toxoplasmosis in cats and dogs. Proceedings of the World Small Animal Veterinary Association. May, 1-14 ,2005; Mexico City, Mexico.
12. Dubey JP, Lindsay DS, Lappin MR. Toxoplasmosis and other intestinal coccidial infections in cats and dogs. Veterinary Clinics Small Animal Practice 2009; 39: 1009-1034. doi: 10.1016/j.cvsm.2009.08.001.
13. Babur C, Erdal N, Biyikoglu G, Piskin FC. Seroprevalence of toxoplasmosis on stray dogs in İstanbul. Acta Parasitologica Turcica 1997; 21: 413-416.
14. Reiter-Owona I, Petersen E, Joynson D, Aspöck H, Darde ML, et al. The past and present role of the Sabin-Feldman dye test in the serodiagnosis of toxoplasmosis. Bulletin of the World Health Organization 1999; 77: 929-935.
15. Kotresha D, Noordin R. Recombinant proteins in the diagnosis of toxoplasmosis. Apmis 2010;118:529-542. doi:10.1111/j.1600-0463.2010.02629.x.
16. Babür C, Altaş MG, Çelebi B, Sevgili M, Taylan Özkan A, et al. Seroprevalance of Toxoplasmosis, Leishmaniosis and Listeriosis in stray dogs in the province of Sanliurfa, Turkey. Turkish Bulletin of Hygiene and Experimental Biology 2007; 64:11-16.
17. Kilic S, Babür C, Özkan AT, Mamak N. Investigation of anti-*Toxoplasma gondii* and anti-Leishmania infantum antibodies among Sivas Kangal Dogs. Turkish Journal of Veterinary and Animal Sciences 2008; 32: 299-304.
18. Gicik Y, Sari B, Babür C, Celebı B. The seropositivity of *Toxoplasma gondii* and Listeria monocytogenes in the dogs of Kars and vicinity. Türkiye Parazitoloji Dergisi 2010 ;34:86-90.
19. Içen H, Babür C, Bademkiran S, Celebi B, Simşek A, et al. Seroprevalance of toxoplasmosis, leishmaiosis and listeriosis in shelter dogs of Diyarbakir, Turkey. Türkiye Parazitoloji Dergisi 2010;34:6-10.
20. Altay K, Babür C, Ataş DA, Beyhan YE, Özkan E. Investigation of seroprevalence of *Toxoplasma gondii* in dogs in the Province of Sivas. Journal of Etlik Veterinary Microbiology 2013; 24:13-16.
21. Doğan N, Özkan AT, Babür C, Köse C. Seroprevalance of leishmaniosis and toxoplasmosis in healthy appearanced street dogs in Eskisehir. Turkish Bulletin of Hygiene and Experimental Biology 2014; 71: 27-34. Doi:10.5505/TurkHijyen.2014.56833.
22. Langoni H, Matteucci G, Medici B, Camossi LG, Richini-Pereira VB, et al. Detection and molecular analysis of *Toxoplasma gondii* and Neospora caninum from dogs with neurological disorders. Revista da Sociedade Brasileira de Medicina Tropical 2012; 45:365-368. doi:10.1590/S0037-86822012000300016.
23. Oliveira TS, Turchetti AP, Barbosa FB, Bicalho AL, Alencar CA, et al. Cutaneous toxoplasmosis in an immunosuppressed dog. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2014; 66: 797-800. doi:10.1590/1678-41626891.
24. Huertas-López A, Sukhumavasi W, Álvarez-García G, Martínez-Subiela S, Cano-Terriza D, et al. Seroprevalence of *Toxoplasma gondi*i in outdoor dogs and cats in Bangkok, Thailand. Parasitology 2021;148:843-849. doi:10.1017/S0031182021000421.
25. Aslan G, Altintaş K. Toksoplazmosis teşhisinde Sabin-Feldman testi ve ELISA IgM antikorlarının karşılaştırılması. Genel Tıp Dergisi 2000;10:161-164.
26. Zarra-Nezhad F, Borujeni MP, Mosallanejad B, Hamidinejat H. A seroepidemiological survey of *Toxoplasma gondii* infection in referred dogs to Veterinary Hospital of Ahvaz, Iran. International Journal of Veterinary Science and Medicine 2017; 5: 148-151. doi:10.1016/j.ijvsm.2017.08.006 .
27. Yan C, Fu LL, Yue CL, Tang RX, Liu YS, et al. Stray dogs as indicators of Toxoplasma gondii distributed in the environment: the first report across an urban-rural gradient in China. Parasites and Vectors 2012; 5: 1-5. doi:10.1186/1756-3305-5-5.