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Araştırma Makalesi / Research Article

Seroprevalence of Canine Toxoplasmosis in Konya province, Turkey*

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Abstract: *Toxoplasma gondii* is a parasitic intracellular protozoan that causes toxoplasmosis in dogs and can infect a variety hosts in the environment. The current study was conducted in Konya province, Turkey, to detect *T. gondii* infection in shelter dogs. Between July 2017 and July 2018, 334 plasma samples were taken from dogs of both genders (males and females) aged 0-1 and 1-3 years. The samples were examined using ELISA to identify *T. gondii*-specific antibodies in plasma using recombinant TgSAG2. Anti-*T. gondii* antibodies were detected in 33.8% of the dogs in the study. Males and females had a seroprevalence of 40.3% and 30%, respectively. No statistically significant difference was observed between the genders. There was also no substantial difference across age groups. Seropositivity rate was 21.4% in 0-1 year old dogs and 34.3% in 1-3 year old dogs. In animals exhibiting clinical symptoms, the seropositivity rate was found to be 36% (22/61). A statistically significant difference was also not found in the infection rates in new and old entry dogs.

Keywords: ELISA, Shelter dogs, Serology, Turkey

Türkiye'nin Konya İlindeki Köpeklerde Toxoplasmosisin Seroprevalansı

Özet: *Toxoplasma gondii* köpeklerde toksoplazmozise neden olan ve farklı konakları da enfekte edebilen hücre içi bir protozoon parazittir. Bu çalışma Konya ilinde barınak köpeklerinde *T. gondii*'nin seroprevalansını belirlemek amacıyla yürütüldü. Temmuz 2017-Temmuz 2018 döneminde 0-1 ve 1-3 yaşlar arasındaki her iki cinsiyetteki köpeklerden toplam 334 plazma örneği toplandı. Plazma örnekleri spesifik *T. gondii* antikorlarını saptamak için rekombinant TgSAG2 tabanlı indirekt ELISA ile analiz edildi. Çalışmadaki köpeklerin %33,8'inde anti-*T.gondii* antikorları tespit edildi. Seroprevalans oranı erkek ve dişi köpeklerde sırasıyla %40,3 ve %30 olarak belirlendi. Cinsiyetler arasında istatistiksel olarak anlamlı bir fark gözlenmedi. Ayrıca yaş grupları arasında önemli bir fark yoktu. 0-1 yaşındaki köpeklerde seropozitiflik oranı %21,4 ve 1-3 yaşındaki köpeklerde ise %34,3 olarak tespit edildi. Klinik semptom gösteren hayvanlarda seropozitiflik oranı %36 (22/61) olarak belirlendi. Yeni ve eski girişli köpeklerdeki enfeksiyon oranlarında da istatistiksel olarak anlamlı farklılık saptanmadı.

Anahtar Kelimeler: ELISA, Barınak köpeği, Seroloji, Türkiye

1. Introduction

Toxoplasma gondii (T. gondii) is an intracellular protozoan parasite that infects all warm-blooded vertebrates, including canines, worldwide. The definitive host cats (Felidae) play an important role in the spread of oocysts, while intermediate hosts are responsible for the parasite's tachyzoite and bradyzoite stages (1, 2). Dogs are considered a mechanical vector or a possible source of toxoplasmosis transmission to humans because they may swallow cat faeces or roll in cat foul-smelling material that contains oocysts. In certain parts of the globe, dog meat is eaten by humans (3, 4). Toxoplasmosis is an uncommon primary illness in dogs, and it is usually caused by immunosuppression and a lack of immunization against the *Canine distemper virus* (CDV). Toxoplasmosis symptoms include broad clinical indicators like fever, anorexia, dyspnea, and more specialized symptoms like neurological, respiratory, cutaneous, or ophthalmic involvement. Cutaneous symptoms are often related to immunosuppression after corticosteroid medication and transplantation. However, the most common coccidian producing skin lesion in dogs is *Neospora caninum*, which should be investigated in the differential diagnosis (32).

Toxoplasma gondii infection in dogs is widespread globally, with prevalence rates varying from 0% to 100% in various regions (1, 5). Akçay et al. (6) reported the first case of canine toxoplasmosis in Turkey as a clinical and histological diagnosis in a dog. Apical complexes, including secretory organelles such as rhoptries (ROPs), micronemes (MICs), dense granules (GRAs), and surface antigens (SAGs, such as SAG1 and SAG2), are present in the invasive stages of apicomplexan protozoan parasites (7). There are four forms of SAG2, two in acute cases (SAG2A, B) and two in chronic cases (SAG2C, D). Tachyzoite expresses SAG2A and SAG2B, which are present throughout the acute phase of the

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illness. During the chronic stage of infection, bradyzoites express SAG2C and SAG2D (8, 9).

Direct, indirect, sandwich, and competitive enzyme-linked immunosorbent assays (ELISAs) are the four primary forms of ELISAs. Different diagnostic performance, including sensitivity and specificity, was found in serum, meat juice, and milk samples, depending on the sample type. Different antigens are used in different ELISAs, including native, recombinant, and chimeric antigens (10). Previously, tachyzoite-based products or complete tachyzoites were utilized as native antigens in ELISAs. ELISA tests based on a recombinant surface protein antigen 2 (rSAG2) have a high sensitivity and specificity that may approach 100%, making them useful as a toxoplasmosis diagnostic tool (11, 12). This research seeks to identify specific antibodies against *T. gondii* infection in shelter dogs at the Municipality of Veterinary Branch Office (Shelter Center) in Konya province, Turkey, utilizing indirect-ELISA based on a recombinant surface protein antigen 2 (rSAG2) as a novel diagnostic tool.

2. Materials and Methods

2.1. Study area

In July 2017, canine blood samples were obtained from the Konya Metropolitan Municipality shelter. The clinical evaluations of 334 dogs of both sexes were documented. In brief, 5 ml of blood was drawn from the ramus dorsal of *Vena cephalica* or *Vena saphena parva* and placed in anticoagulant tubes. The blood was then centrifuged at 2500 rpm for 15 min to separate the plasma, then kept in a deep freezer (-20°C) until used. Indirect ELISA was used to determine plasma *T. gondii* specific IgG antibodies at Selcuk University, Faculty of Veterinary Medicine, Department of Parasitology Laboratory, Turkey. 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. In vitro production of the recombinant SAG2 antigen (rTgSAG2)

The ELISA relies on recombinant TgSAG2, generated from tachyzoites in Obihiro University, Faculty of Agriculture and Veterinary Medicine, National Research Center for Protozoan Diseases Laboratory in Japan, then imported to Turkey and utilized as an antigen in the ELISA test. The recombinant TgSAG2-GST protein utilized in this study was generated using the previously reported approach (13). The PCR product of the truncated TgSAG2 gene was transformed into the *E. coli* BL-21 strain following insertion into the DNA of the pGEX-4T vector (Amsterdam Pharmacia Biotech, San Francisco, CA., U.S.A). By providing the optimum in vitro growth media for transformed *E. coli* BL21 cells, the TgSAG2 protein was induced to express. In brief, 10 ml of newly transformed *E. coli* cells were cultivated at 37°C at 250 rpm in 1L LB media with 50 µg/ml ampicillin until the optical density (OD) at 600 nanometers reached 0.5. The recombinant SAG2 antigen expression was stimulated using 5 mM isopropyl-D-1-thiogalactopyranoside (IPTG) and incubated overnight at 27°C, after which the cells were continued to proliferate. The culture of *E. coli* was centrifuged at 8,000 g for 15 min and then the cell pellet in TNE buffer (50 mM Tris-HC1, pH 8.0, 100 mM NaCl, two mM EDTA, and 1% Triton X-100), 50 mg/ml lysozyme reconstituted in a buffer containing 1% (w/v) N-Laurosylsarcosine sodium and protease inhibitors. This recombinant protein was purified using Glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech).

2.3. Indirect ELISA assay

A 40% BCA protein kit was used to determine the concentration of expressed proteins. TgSAG2-GST proteins were diluted in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) at a 2-4 µg/ml concentration for the indirect ELISA assay. The ELISA plate wells were coated with 100 µl of antigen and incubated at 4°C overnight. After pouring the coating solution, the plates were blocked for 1 h at 37°C with PBS containing 3% skim milk powder. Following a PBST wash, serum samples (diluted 1:100) were added to the plates and incubated at 37°C for 1 h. Horseradish peroxidase (HRP)-bound anti-dog IgG (1:4000) (Bethyl, Montgomery, AL, USA) was added, and it was followed by ABTS [2,2'-azinobis (3-ethylbenzthiazolinesulfonic acid)] (Sigma, USA, Louis, MO, USA). The colour development was seen at room temperature, and 50 µl of stop solution (2 M Sulfuric acid) was added. A microplate reader (ELISA reader/Rayto Microplate Reader, Model: RT-2100C) was used to measure optical density (OD) at 415 nm. The ELISA results were evaluated for each sample by subtracting the GST protein's cut-off value of OD = 415 from the recombinant TgSAG2's OD = 415 value. Cut-off values were calculated using negative dog serum samples by multiplying the mean value of negative sera measured at OD = 415 by three times the standard deviation. When the value of the tested sample exceeded the cut-off value, the sample was considered positive.

2.4. Clinical manifestations and co-infection

Various clinical symptoms, including hindlimb paralysis and atrophy, skin lesions, nasal discharges, vomiting, diarrhoea, tick infestations, weakness, and nervous system abnormalities, were recorded.

2.5. 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.

3. Results

The overall infection rate in the 334 dogs examined was 33.8%. Males accounted for 40.3% of the infection, while females accounted for 30%. The distribution of infection according to genders is indicated in Table 1 and Figure 1.

Table 1: Distribution of infection according to genders

Gender	Number of animals	(-)	(+)	%
Male	124	74	50	40.3
Female	210	147	63	30
Total	334	221	113	33.8
		(P>0.05)		

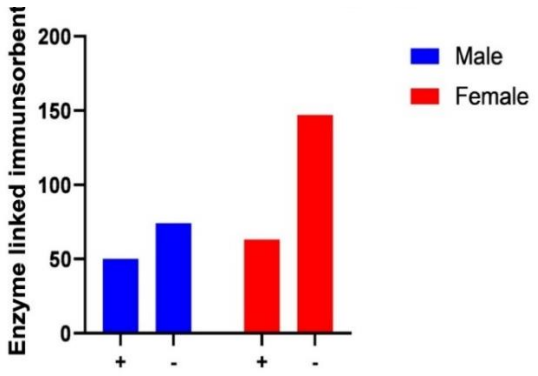

Figure 1: The number of seropositive dogs according to genders

The study included 334 dogs of both genders from the Konya Metropolitan Municipality Veterinary Branch Directorate (Shelter Centre). General clinical results for all dogs were recorded based on age and gender. Anticoagulant tubes were used to collect blood samples (3-5 mL), and plasma was separated for serological analysis. The ELISA detection of *T. gondii* antibodies is based on recombinant SAG2 protein. Infection status by age is shown in Table 2 and Figure 2.

Table 2: Distribution of infection according to ages

Ages	Number of animals	(-)	(+)	%
0-1	14	11	3	21.4
1-3	320	210	110	34.3
Total	334	221	113	33.8
		(P>0.05)		

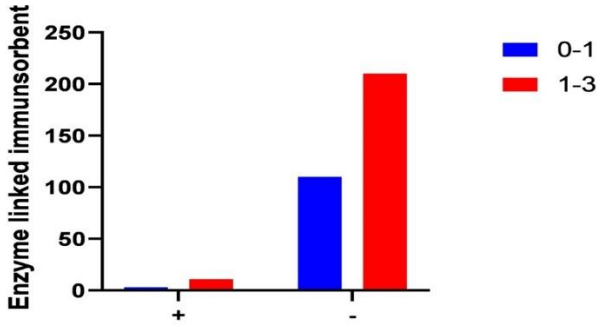

Figure 2: The number of seropositive dogs according to ages

Seropositivity was 21.4% (3/14) in the 0-1 age group and 34.3% (110/320) in the 1-3 age group. This research documented various clinical symptoms, including hind limb paralysis and atrophy, skin lesions, nasal discharges, vomiting, diarrhea, tick infestations, weakness, and nervous system disorders. The relationship between ELISA and clinical results is shown in Table 3 and Figure 3.

Table 3: The relationship of the infection with clinical symptoms

Clinical symptoms	Number of animals	(-)	(+)	%
A	1	0	1	100
B	32	22	10	31.2
C	18	11	7	38.8
D	9	6	3	33.3
E	1	0	1	100
Total	61	39	22	36
		(P>0.05)		

A: Hindlimbs paralysis and atrophy; **B**: Nasal discharges; **C**: Skin lesions; **D**: Tick Infestations; **E**: hindlimbs paralysis and atrophy and skin lesions

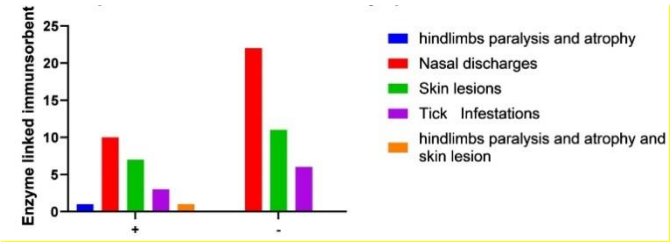

Figure 3: The number of seropositive dogs according to clinical symptoms determined in dogs

Different clinical signs were shown in 36% (22/61) of the dogs. Some animals were very seropositive, with 100% (1/1) having hind limb paralysis and atrophy, 38.8% (7/18) having skin lesions, 33.3% (3/9) having tick infestation, 31.2% (10/32) having nasal discharges, and 100% (1/1) having hind limb paralysis, atrophy, and skin lesions. The seropositivity status of new and old entrance dogs was determined using an ELISA test. Seropositivity was

identified in 14/44 (31.8%) of freshly entered dogs and 99/290 (34.1%) of dogs with previous entrance (Table 4 and Figure 4).

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

Status type	Number of animals	(-)	(+)	%
New entries	44	30	14	31.8
Old entries	290	191	99	34.1
Total	334	221	113	33.8
		(P>0.05)		

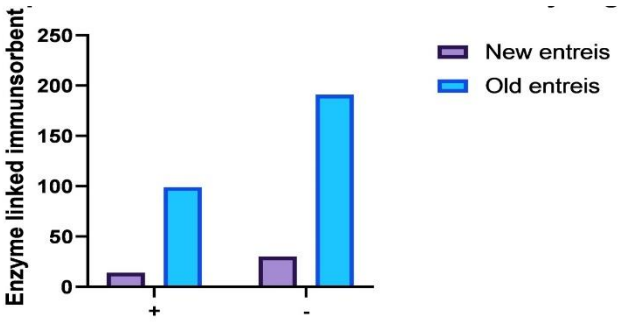

Figure 4: The number of seropositive dogs according to new and old entry dogs

4. Discussion and Conclusion

Toxoplasma gondii is a coccidian obligate intracellular protozoan parasite capable of infecting almost all warm-blooded animals, including humans and dogs (1). Canine toxoplasmosis is a very uncommon primary illness with clinical signs (5, 14). 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 (15-18). Numerous recombinant proteins from *T. gondii* have been produced and characterized for sensitivity and specificity in IgG ELISA, the most often used serological test (10). ELISA was created to detect different kinds of antibodies or antigens (19) and is commonly used to diagnose *T. gondii* infection. Due to its tremendous sensitivity and specificity, it is inexpensive, quick, and uncomplicated (17, 32). The study included 334 canines and was done by the Veterinary Branch Directorate of the Konya Metropolitan Municipality. This reseach aims to use an indirect ELISA test in Konya to identify the specific anti-*T.gondii* antibody. Gender, age, clinical symptoms, new and old entrances, water supply, shelter cat discovered, feed nature, and rodent (rat) distribution were all documented at the Shelter centre throughout the experiment. When ELISA was used to test recombinant TgSAG2, 33.8% of all seropositives were identified in this investigation. Other researchers have come to the same conclusion (16, 19, 20). Various variables may influence ELISA results, including the type of ELISA (direct, indirect, and commercial ELISA), laboratory conditions, substrates, and other procedures. Males were infected at a rate of 40.3%, while females were infected at 30%; this study's high seropositivity implies considerable environmental contamination (16). *T. gondii* was detected by serology in stray or free-living dogs in China. ELISA revealed that 40.3% of 231 stray dogs, 38.7% of urban dogs, and 41% of rural dogs were infected, with no statistically significant difference between infections. While there was no statistically significant difference in frequency between the under-one-year age group (21.4%) and the 1-3 age group (34.3%), there was no significant difference in frequency within the under-one-year group (21.4%). The sample size was limited, and the seroprevalence of cases was 3/14 (21.4%) in 0-1-year-old animals, as indicated in this study. Future research should address these issues. These results agree with the studies conducted by Oncel et al. (33) and Kilic et al. (34).

The distinction explains how and when high-age animals may get infected throughout their lives. The findings were in accordance with those of (22) who used a commercial indirect-ELISA to determine the seroprevalence of *T. gondii* infection in 456 domestic dogs in Panama. There were 147 people who tested positive for IgG antibodies (32.23%). Consequently, there was a significant prevalence of canine toxoplasmosis in this area, which increased with age. The severity of symptoms is unrelated to the antibody titer. The detection of circulating *T. gondii* antigens may potentially help with toxoplasmosis diagnosis (5, 23). However, in this investigation, recombinant proteins were utilized as antigens to detect circulating antibodies. The majority of dogs have neuromuscular, respiratory, and gastrointestinal symptoms (5). The majority of clinical toxoplasmosis signs in older dogs are linked to cerebral indications of neosporosis. Despite the differences in these illnesses, the symptoms are comparable (1). In Brazil, a seven-year-old female dog was found with cutaneous ulcerated nodular lesions. Multiple bradyzoite cysts and tachyzoite panniculitis and dermatitis, were discovered during the biopsy (24). In an ELISA test, 22 (36%) of 61 canines with clinical symptoms were seropositive. Other symptoms mentioned included vomiting, diarrhoea, neurological system abnormalities, and weakness. The findings are similar to previous findings, which varied from 1.5 to 100% in various canines (1, 5, 15, 24-28). Previous research suggests that *T. gondii* infections have been fluctuating at different rates. Different habitats of dogs, differences in age and gender, strain, and diet, a close relationship with the final host (cat) or reservoir hosts (such as rodents) involved in the transport, the absence or presence of other infections during the examination, the immune systems of the animals, and differences in the tests used for diagnosis could all contribute to differences in infection rates. Other factors include sample size, ELISA type (direct

vs indirect), test location, and immune-suppressive illnesses, such as canine distemper virus.

Seropositive in ELISA were 22 (36%) out of 61 dogs showing clinical signs. Other clinical signs like vomiting, diarrhoea, nervous system disorders, and weakness were reported. The results are consistent with others (5, 15, 23-27).

Seropositivity was determined in 14 (31.8%) of 44 newly admitted dogs and 99 (34.1%) of 290 dogs with old entry. This result agrees with other results (13, 16, 18, 20, 24). Results from previous studies suggest that *T.gondii* infections have been varying at rate infections. Differences in infection rates may be caused by different habitats of dogs, differences in age, strain, and diet, close relationship with the final host or reservoir hosts involved in the transmission, co-infections, immunity, and tests used for diagnosis.

In conclusion, in Turkey, shelter dogs are more susceptible to infection from meat or water, while contaminated foods increase the risk of human toxoplasmosis. *T. gondii* is a zoonotically important protozoan parasite that can be transmitted in many different ways and should be paid attention especially by veterinarians. It is required to take various measures to reduce the danger of toxoplasmosis and to prevent *T. gondii* infections.

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