

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN DOGS IN EASTERN TURKEY

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ABSTRACT

This study investigated the seroprevalence of *Toxoplasma gondii* in dogs in Erzurum, the largest province of Eastern Turkey which until now had no animal shelter. Sera from 72 stray dogs that presented to the shelter (44 female and 28 male) were examined by Sabin-Feldman Dye Test for antibodies to *T. gondii*. Seventy sera (97 %) were found to be positive. Among the seropositive dogs, 82% were one year old or younger while all the older dogs were seropositive. Seropositivity was found to be 100 % in males and 95.5 % in female dogs.

Keywords: Dog, Eastern Turkey, Sabin-Feldman, Seroprevalance, *Toxoplasma gondii*

INTRODUCTION

Toxoplasmosis is a parasitic zoonotic disease caused by the obligate intracellular protozoa *Toxoplasma gondii* (1). *T. gondii* was described by Charles Nicolle and Louis Manceaux for the first time in 1908 in North African rodents known as "gundi", hence the name "gondii" (2). In dogs *T. gondii* infection were reported by Mello for the first time in 1990 (3, 4).

Cats are the definitive host for *T. gondii* which has the ability to infect humans and other warm blooded animals (3). Humans and animals are infected by eating raw or undercooked meat containing cysts or by the ingestion of food which is contaminated with sporulated oocysts (5). Although *T. gondii* does not undergo an enteroepithelial cycle in dogs, the fact that it is observed more frequently in people who are in contact with dogs rather than cats calls attention to the importance of dogs as a source of infection (6).

The clinical symptoms of toxoplasmosis in dogs are generally characterized by respiratory impairment, diarrhoea and ataxia (7). The disease may be suspected from clinical findings however, laboratory methods are required to confirm the diagnosis. There are a number of serologic tests used for *Toxoplasma* diagnosis such as the Sabin-Feldman dye test (SF), complement fixation (CF), indirect hemagglutination assay (IHA), enzyme linked immunosorbent assay (ELISA), indirect fluorescence antibody test IFAT and the latex agglutination test (LAT). Among these the SF dye test has been found to be both specific as the most sensitive (8, 9).

A number of surveys have been carried out to determine the seroprevalance of *T. gondii* infection globally (10) and in

Turkey (1, 2, 11-15). The aim of this study was to investigate the seroprevalance of *T. gondii* with the SF dye test in sera of dogs from an animal shelter in the Erzurum province, in Eastern Turkey, and to determine the effect of age and gender on seropositivity.

MATERIALS AND METHODS

Sample collection

Sera of a total of 72 dogs were obtained from an animal shelter of the Erzurum municipality in Eastern Turkey. Ethical approval for this study had been obtained from Erzurum Veterinary Control and Research Institute. Forty four female and 28 male dogs were involved in the study. The mean age of dogs was 2.8±1.3 (minimum 0.5 year old and maximum 6 years old). Ten millilitres was collected from the cephalic vein transferred into vacuum tubes. Samples were centrifuged at 3000 rpm for 10 minutes; the sera were separated and stored at -80 °C until testing.

Serologic examination

All sera were examined for *T.gondii* antibodies using the SF dye test as described (16). The examinations were carried out at the reference laboratory, Ankara Refik Saydam Hygiene Center.

Healthy 3–4 weeks aged white Swiss albino mice were used for the preparation of test antigen. *T. gondii* Rh antigen was derived from the peritoneal fluid of these mice 48 hours after injecting them with a virulent strain. As an activator serum,

human serum (known to be seronegative for *T. gondii*) was used. Alkaline methylene blue dye was prepared with 9.73 ml of 0.53 % Na_2CO_3 (Sigma, Seelze, Germany), 0.27 ml of 1.91 % $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (Merck, NJ, USA) and 25 mg of methylene blue (Difco, Detroit, MI, USA). Following inactivation of complement at 56 °C for 30 minutes, 25 µl of test sera were prepared with normal saline in dilutions of 1:4, 1:16, 1:64, 1:256 and 1:1024. Antigens in 25 µl activator serum and approximately 25 *T. gondii* tachyzoites on a microscopic field of 40x magnification were added to the sera preparations. The mixture was incubated in a water-bath at 37°C for 50 minutes. 50 µl of alkaline methylene blue was added to the mixture and kept in 4 °C for 10 minutes. *T. gondii* stained tachyzoites were examined under light microscope with 40X objective. If more than 50 % of tachyzoites on one microscopic field were not stained, this dilution step was accepted as positive. Titers of 1:16 and above were considered as positive. Positive and negative controls previously confirmed by IFAT method were included in the above procedure.

Results were presented as percentages and a Chi-square test was used for statistical analysis and considered to be significant at $p < 0.05$.

RESULTS

Anti-*T.gondii* antibody was detected in 70 (97%) of 72 sera of dogs that were examined by using the SF dye test. Thirteen dogs (18.5%) were seropositive at titer of 1:16; 46 dogs (65.7%) were seropositive at titer of 1:64 and 11 dogs (15.7%) at a titer of 1:256. Seropositivity was 100% in male dogs and 96% in female dogs.

When the results were analyzed according to age seropositivity was 82% for dogs one year old or younger and 100% in older dogs. Table 1 summarizes the results of the relationship between seropositivity and age and gender. Seropositivity of *T.gondii* was detected in a higher percentage of male dogs than female dogs; however, this difference was not found to be statistically significant ($p > 0.05$), while the difference between the ages was statistically significant ($p < 0.05$).

DISCUSSION

T. gondii is an obligate intracellular parasite causing toxoplasmosis which is commonly observed in humans and animals (3, 17). The incidence of toxoplasmosis in dogs has been reported throughout the world at percentages varying between 0 and 100% (18-21). *T. gondii* has been detected histopathologically by Akcay and others (22) for the first time in Turkey and its isolation was carried out by Ekmen and Altintas (23). Numerous studies have been performed in dogs to investigate the prevalence of toxoplasmosis in different regions of Turkey (1, 11, 13, 15, 24, 25). To the best of the authors' knowledge, this study is the first performed for the determination of *Toxoplasma* seroprevalance in dogs in the Erzurum province.

The clinical findings of toxoplasmosis in dogs are generally

characterized by respiratory impairment, diarrhoea and ataxia. The infection is more frequently seen in puppies rather than in older dogs and is generally accompanied by infection with canine distemper virus. Focal necrotic areas are generally present in the liver, lungs and brain of dogs with toxoplasmosis resulting in the development of different clinical syndromes (7). Clinical definitive diagnosis is difficult to make due to the fact that the course of the disease may be subclinical and asymptomatic. The SF test was used in the study as it is considered a highly sensitive test.

Serological studies have been performed on dogs in Turkey using methods other than the SF test. Using the LAT, seropositivity values were detected in 49% of dogs in Ankara and in 85% of dogs using the IFAT (1). In Konya 67% of dogs were positive by the IFAT while 59% were positive using the Modified Agglutination Test (MAT) (25). In Aydin and Bursa, 28% and 17%, respectively were seropositive using the IFAT (2, 14) and in Van, 10% of dogs were seropositive using an ELISA test (12). As reported, higher seropositivity rates were obtained with the SF test compared to other tests. Nevertheless, high *T. gondii* seropositivity rates in dogs were found in all tests performed.

In the studies performed using the SF dye test for the serological diagnosis of toxoplasmosis in dogs in various regions of Turkey, seropositivity values were observed at the following rates in the following towns; 64 in Konya (25); 72% in Istanbul (26); 75% in Elazig (11); 69% in Bursa (15); 60% in Aydin (13); 70% in Kocaeli (27); and 62 % and 79% in Ankara (1, 28). This study detected positivity in 97% of dogs tested in the Erzurum. The probable reasons of the percentage differences in the studies may be related to geographic and lifestyle conditions and the variations in the environmental conditions. Furthermore the higher percentages in the present study may be due to the fact that these are stray dogs in close contact with cats with the possibility of fecal oocyst exposure occurring. Furthermore, stray dogs have been observed to feed from disposed meat from abattoirs in the area.

No previously studies have been performed in Erzurum for seroprevalance of *T. gondii* in dogs. In 1975, Altintas (29) investigated toxoplasma antibodies by using the SF dye test in 603 sheep which were taken from various regions of Turkey and brought to Ankara's Meat and Fish Authority for slaughtering. Sheep arriving from Erzurum had a seropositivity ratio of 31 %. Yigit and others (30) found seropositivity for toxoplasmosis in 24 % of people at the Erzurum Ataturk University Medical Faculty. The high seropositivity of *T. gondii* in dogs from Erzurum in the present study raises the possibility that dogs may present a risk to humans in this region.

Seropositivity comparing male and female dogs was determined in other studies in different regions of Turkey. In Elazig and Ankara equal numbers of males and females were seropositive while in Aydin and in the Gemlik Military Stud more females than males were seropositive (2, 11, 15, 28). The present study results are comparable with those of Aydin and in Gemlik. The reason for the higher percentage of seropositivity

among female dogs is unclear but may be due to the greater number of females sampled in the present study.

The relationship between the ages of the dogs and their seropositivity for *T. gondii* have been studied and found to vary considerably. Inci and others (1996), determined that the highest seropositivity (81%) was in the age group of 3 months to one year of age; Handemir and others (2001) found higher seropositivity ratio (23%) in dogs older than 5 years. Aktas and others (1998), showed that dogs older than one year had a seropositivity ratio of 80 % whereas Aslantas and others (2005), suggested that this percentage was associated with the age group of 3-5 years. Eren and others (2002), determined the highest ratio of seropositivity of 40 % in the age group of 4 years. The reason for the differences found between other studies and the present study are not clear but may be related to environmental and geographic factors.

In conclusion, seropositivity of dogs *T. gondii* has been found to be at a relatively high ratio in the Erzurum province. This leads to the consideration that the high rate of infection among dogs may pose a serious human and animal risk and that prophylactic measures should be taken to prevent further spread of the disease.

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Table 1. Seroprevalance of *Toxoplasma gondii*, percentage of positivity and antibody titers of dogs by sex and age.

Sex / Age	Number of Sera	Number of Positive Samples	Percentage of Positivity (%)	Titers 1:16	Titers 1:64	Titers 1:256
Male	28	28	100	6	21	1
Female	44	42	94	7	25	10
≤1 year-old	11	9	82	2	5	2
>1 year-old	61	61	100	11	41	9