

Seroprevalence of Toxoplasmosis in Donkeys in Eastern Turkey

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ABSTRACT

The aim of this study was to investigate the seroprevalence of *Toxoplasma gondii* in donkeys in Erzurum, the largest province of Eastern Turkey. The current study was carried out on serum samples collected from 55 female and 37 male donkeys from the Erzurum province which were examined using the Sabin Feldman Dye test. Out of 92 collected samples, 57 (62%) of sera were found to be seropositive at the dilution of 1:16 and greater. The rates of positivity for female and male donkeys were 67.3% and 54.1%, respectively. As far as age groups were concerned, 38.6% positivity was found at 0-3 years, 50.9% at 4-6 years and 19.3% at 7 years of age or older. This study indicated that toxoplasmosis in donkeys is common in Erzurum province and displays different ratios in terms of age and gender.

Keywords: Donkey, Eastern Turkey, Sabin-Feldman, Seroprevalence, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii is a significant zoonotic protozoan that may infect birds and mammals, including humans. Infection of *T. gondii* is subclinical in equines. Serological tests are generally employed in the diagnosis of the disease. Although a small number of patients display clinical symptoms, antibody seropositivity has been reported to be quite high (1, 2).

There have been a number of studies conducted on equines from various countries. Dubey (3) isolated *T. gondii* from tongue, intestine, heart, eyes and lung in his experimental studies. Turner and Savva (4) detected *T. gondii* DNA in the eyes of horses using PCR method. Seropositivities ranging between 1% and 37% have been reported in a variety of studies conducted on equines in different countries (5, 6). *T. gondii* antibody levels in equines were determined using different serological tests in various parts of the world (7-10). In China (8), 1.51% of horses seropositive for *T. gondii* antibodies were reported using the indirect haemagglutination (IHA) test; in Argentina (9), 20% of horses had antibodies

to *T. gondii* and in Brazil (10), seropositivity was detected in 17% horses. In serological studies conducted in the Czech Republic, *T. gondii* antibodies were found to be 7.7% and 4.1% using the Sabin Feldman Dye Test (SFDT) and the Complement Fixation (CF) Test, respectively (7). Dubey (11) reported seropositivity in 17.4% of horses in North America using SFDT. The studies conducted in USA and Nigeria by using the Indirect Haemagglutination test (IHA), on the other hand, indicated 20% and 37.1% seropositivity respectively (5, 12). In studies conducted in Argentina and North America using Modified Agglutination Test (MAT), 13.1% and 6.9% seropositivity was reported, respectively (11, 13). Seropositivity rate of 1% was obtained in horses in Sweden (6) using ELISA, whereas seropositivity rate of 2.6% was obtained in South Korea (14) using IFAT.

The number of studies available in Turkey on the seroprevalence of *T. gondii* cover a number of areas in the country. In 1970, Weiland and Dalchow (15) reported the presence of toxoplasmosis in horses for the first time in Turkey. They

found 14.3% seropositivity among 154 horses using SFDT. Since then, *T. gondii* seropositivity has been studied in many regions of Turkey and seropositivity ranging between 1.8% and 42.2% has been reported (16-25). Using SFDT, Zeybek *et al.* (26) detected 8.2% *T. gondii* seropositivity in horses and 24% seropositivity in donkey serums collected from various regions of Turkey and determined the presence of seropositivity in females and males to be 6.0% and 9.9% respectively. The same samples were studied using Latex Agglutination Test (LAT). *T. gondii* seropositivity was detected as 11% in donkeys and 6.2% in horses, of which 24.1% *T. gondii* positive animals were females and 23.6% were males.

In a study conducted in the Kayseri region by Inci *et al.*, 23 (19.2%) of 120 equines were found to be seropositive for *T. gondii* using SFDT (22). Distribution in animals was reported to be 42.4% of donkeys, 10.4% of horses and 10% of mules. Of 23 seropositive animals, 65.2% were at a dilution of 1:16, 17.4% at 1:32 and 17.4% at a dilution of 1:64. Likewise, Inci *et al.* (23) detected 1.9% seropositivity in another study conducted using SFDT on horses in Gemlik Military Stud Farm.

Using SFDT, Babur (20, 21) reported 8.33% and 2% seropositivity in equines in Ankara; Aktas *et al.* (18) reported 6.4% in Malatya; Akca *et al.* (16) reported 20.6% in Kars; Sevgili *et al.* (24) reported 7.5% in Sanliurfa. In a study conducted in Van using IHA, 1.7% seropositivity was detected in horses (17). In another study conducted by Guclu *et al.* (27) on horses bred for sport purposes in the province of Ankara, sera collected from a total of 100 horses were tested using SFDT and 28% were found to be positive. Seropositivity was reported to be 82.1% at a dilution of 1:16 and 17.8% at a dilution of 1:64. In the Nigde region 7% seropositivity was detected among horses using the SFDT (28).

The objective of this study is to determine the incidence of seropositivity against *T. gondii* in donkey sera collected at random in the province of Erzurum. The sera were tested using the SFDT for serological diagnosis of toxoplasmosis.

MATERIALS AND METHODS

Sample collection

The blood samples were randomly collected from 92 donkeys consisting of 55 females and 37 males, within the province of Erzurum and its countryside. Ethical approval for this study was obtained from the University of Ataturk (AUHADYEK)

animal care and use committee. Using information obtained from the animal owners, 42 of these donkeys were in the age range 0-3 years, 39 of them were in the age range 4-6 years and 11 of them were 7 years and older.

The blood samples were collected from the vena jugularis of the donkeys and transferred into vacuum tubes. The blood samples were centrifuged at 4000 rpm for 10 minutes at room temperature. Subsequently they were placed into eppendorf tubes and stored at -20°C until use.

Serologic examination

All sera were examined for *T. gondii* antibodies using the SF dye test as described (29). The examinations were carried out at the reference laboratory, Ankara Refik Saydam Hygiene Center.

The procedure included two steps in preparation for performing the test. Healthy 3–4 week old white Swiss albino mice were injected with the virulent RH strain of *T. gondii*. *T. gondii* RH antigen was collected from the peritoneal fluid of mice after 48 hrs post injection. As an activator serum, human serum seronegative for *T. gondii* was used including factors such as magnesium, properdin, C2, C3, C4. Alkaline methylene blue dye was prepared with 9.73 ml of 0.53% Na₂CO₃ (Sigma, Seelze, Germany), 0.27 ml of 1.91% Na₂B₄O₇·10H₂O (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. Antigen within 25 µl activator serum, approximately 25 *T. gondii* tachyzoites in a microscopic field of 40x magnification, was 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. Examination was carried out under light microscope with 40X objective to gauge whether *T. gondii* tachyzoite were stained. 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 (30). Positive and negative controls which were confirmed by IFAT method were included in the above procedure to evaluate the reliability of the test.

Statistical analysis was carried out using the Kluskal Wallis test for non-parametric data and P≤0.05 was considered as significant.

RESULTS

Anti-*T. gondii* antibodies were detected in 57 of 92 (62%) donkeys by using SFDT test. Forty three (75.4%) of donkey sera were detected to be seropositive at 1:16 dilution and 14 (24.6%) were seropositive at 1:64 dilution. Seropositivity rate was determined to be 54.1% in male donkeys and 67.3% in female donkeys. The difference between the seropositivity of males and females was not statistically significant.

Analyzing the results for age groups, 38.6% seropositivity rate was determined in donkeys between the age of 0 and 3 years; 50.9% between 4-6 years and 19.3% of donkeys 7 years and older. Although differences were noted between the different ages no statistical significance was present. The relationship between positive results for gender and age groups is presented in Table 1.

Table 1: Seroprevalance of *Toxoplasma gondii* and percentage of positivity over sex and age in donkeys

		Number of Sera	Positive Samples	
			(number)	(%)
Sex	Male	37	20	54.1
	Female	55	37	67.3
Ages (years)	0-3 years	42	22	38.6
	4-6 years	39	29	50.9
	7 years and over	11	6	19.3
Overall		92	57	62.0

DISCUSSION

Toxoplasmosis is a zoonotic infection of both humans and animals and especially of importance for humans in contact with animals and animal products (31, 32). SFDT, used in this study, is considered as a precise test in the diagnosis of toxoplasmosis and may be employed as a reference serological test (7, 11).

This is the first study intended to determine the *T. gondii* seroprevalance in donkeys within the region of Erzurum where 57 animals (62%) of 92 animals, were found to be seropositive using SFDT. It is notable that this result is higher than results obtained previously using the same test (16, 18, 20-24, 26-28).

In a study conducted on horses in Nigde region it was reported that the prevalence in both age groups tested were similar for 1-10 years of age and 11-20 years of age (28), The relationship between the test results and age groups was ana-

lyzed, and 7.4% seropositivity was observed in the age group of 1-10 years and 6.8% seropositivity was observed in the age group of 11-20 years. Analysis of the results by age revealed that the highest seropositivity was observed in donkeys of 4-6 years of age. The high of seropositivity in this age group may be due to the accumulative exposure of these animals over time. Thus older animals in the same area may have been presumed to have an increased seroprevalance. None the less, this difference between age groups was determined to be statistically insignificant ($P > 0.05$).

In this study, 92 donkey serums were analyzed using SFDT, 43 of which (75.4%) were found to be seropositive at 1:16 titration and 14 of which (24.6%) were found to be seropositive at 1:64 titration. When these results are compared with the results of studies previously conducted in different regions (22, 26-28), percentage differences at low levels are observed; however, a similarity of results attracted attention since the maximum seropositivity rate in this study was at 1:16 titration as in other studies.

In conclusion, it was found *T. gondii* seroprevalance is at a high rate in donkeys in the province of Erzurum. Depending on gender and age different distributions were detected, however no statistical differences either for age or gender of the donkeys were found.

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