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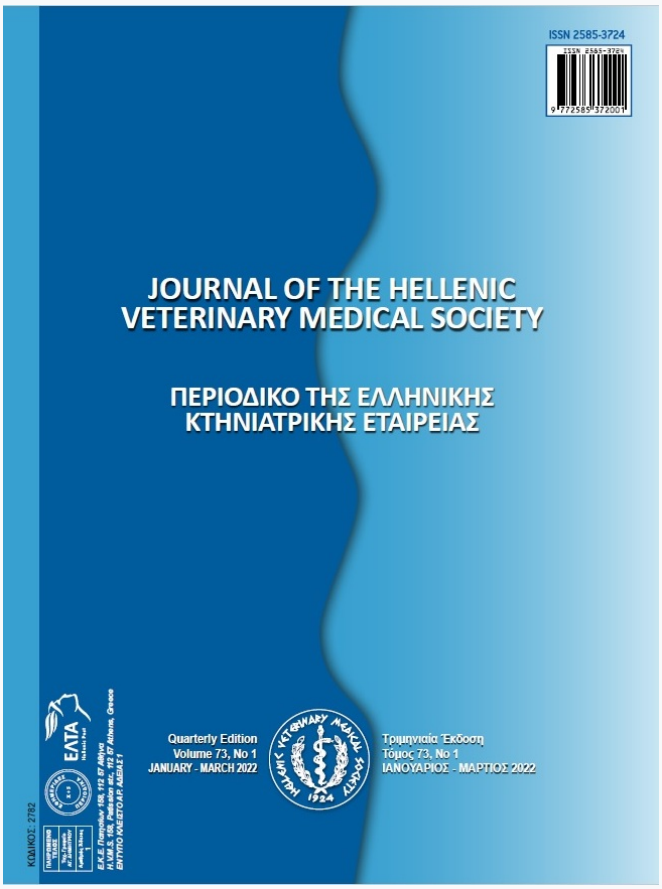

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Research article
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Investigation of Seroprevalence of Toxoplasmosis in Horses and Donkeys in Muş Province of Turkey*

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ABSTRACT: The purpose of this study was to determine the seroprevalence of Toxoplasmosis in equidae in the province of Muş of Turkey. The study material consisted of 210 equidae including 159 horses and 51 donkeys in Muş province. In serum samples, *anti-Toxoplasma gondii* antibodies and titers were detected using Sabin Feldman Dye Test (SFDT). Seropositivity was found in 115 (54.76 %) of the 210 equidae tested in the study. The rate of seropositivity in donkeys (92.16%) was higher than the rate in horses (42.77%), and statistical significance was observed (P<0.001). *T. gondii* antibody was detected in 68 (42.77%) of the horse sera. When *T. gondii* seropositivity was evaluated according to gender, it was found to be 47.92% in females and 32.92%in males. No statistical difference was observed between the gender groups (P> 0.05). When *T. gondii* seropositivity was evaluated according to age, seropositivity rate in those older than 10 years was found to be higher as 46.67%, but no statistical significance was observed among the age groups. *T. gondii* antibody was detected in 47 (92.16%) of donkey sera. When *T. gondii* seropositivity was evaluated according to gender, the rate of seropositivity was found to be 89.47% in females and 93.75% in males. No statistical significance was observed between the gender groups (P>0.05). When *T. gondii* seropositivity was evaluated according to age, the seropositivity rate in those older than 10 years was found to be higher as 96.30%, but no statistical significance was observed among the age groups (P>0.05). As a result of scanning the equidea in Muş province by SFDT, seropositivity rate was found as 42.77% in horses and 92.16% in donkeys.

Keywords: Donkey, Horse, SFDT, *Toxoplasma gondii*, Toxoplasmosis

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INTRODUCTION

Toxoplasmosis is a zoonotic, protozoal disease caused by the *Toxoplasma gondii (T. gondii)* agent (Dubey and Beattie, 1988). *T. gondii* has a wide variety of intermediate hosts from warm-blooded animals to humans. The agent is zoonotic in character and is an obligate intracellular protozoal parasite of feline (Garcia-Bocanegra et al., 2012).*T. gondii* is estimated to infect the population with a worldwide distribution, and to the extent that if it infects human, it is of great threatto animal health as well owing to its wide range of transmission (Kim and Weiss, 2008). Although *T. gondii* infection is subclinical in equidae, it may show symptoms such as fever, ataxia, retinal degeneration, encephalomyelitis, and may also lead to abortion or stillbirth (Güçlü et al., 2007; Miao et al., 2013). The disease is more severe in young and immunosuppressed animals (Güçlü et al., 2007; Kar and Güven, 2016).

To diagnose toxoplasmosis, serological tests such as Sabin-Feldman Dye Test (SFDT), Modified Plate Agglutination Test (MAT), Indirect Fluorescent Antibody Test (IFAT), Indirect Hemagglutination Test (IHAT), Enzyme-linked Immunosorbent Assay (ELISA), Immunosorbent Agglutination Assay (ISAGA), Lam Aglutination Test (LAT), Piezoelectric Immunoagglutination Assay (PIA), Western Blot (WB), Immunochromatographic Assay (ICT) and Avidity Test are applied (Liu et al., 2015).

Studies on investigation of the seroprevalence of toxoplasmosis in equidae (horses and donkeys) have been conducted in various region of Turkey. However, no study has been carried out in Muş province of Turkey. Therefore, in this study was aimed to determine the presence of *anti-Toxoplasma* antibodies and the seroprevalence of *T. gondii* by using SFDT in equidae from this province.

MATERIALS AND METHODS

Animals and sample collection

This research was carried out in accordance with the Sub-Committee Decision No: 2017/13 of Ataturk University, Faculty of Veterinary Medicine. The present study was conducted on 210 equidae (159 horses and 51 donkeys) which were randomly selected regardless of age and gender in Muş Province of Turkey (Table 1 and Table 2).The blood samples collected from the *vena jugularis* of the horses and donkeys were centrifuged at 3000 rpm, the sera were separated and stored at -80^{0}C until analyzed by the serological tests.

Serological examination

Serological tests of sera were performed by SFDT at the Parasitology Laboratory of Refik Saydam Hygiene Institute (Ankara, Turkey) (Sabin and Feldman, 1948). For the production of live antigen, *T. gondii* seronegative *Mus musculus* albino white mice ranging in age from 3-6 weeks were tested. For the continuation of live antigen production and the use of live antigen in tests, 2 ml of diluted liquid was produced by diluting with 0.9% Sodium Chloride (NaCl) solution to contain approximately 15-16 *T. gondii* tachyzoites in field controls performed *T. gondii* tachyzoites in field controls performed under the light microscope (10×40). Each mouse

Table 1. Foci, species and gender distribution of the examined group

Study region	Horse	Female horse	Male horse	Donkey	Female donkey	Male donkey	Total
Muş (Center)	73	45	28	25	4	21	98
Bulanık	18	11	7	19	8	11	37
Haskoy	35	21	14	1	1	0	36
Korkut	3	3	0	1	1	0	4
Malazgirt	20	10	10	5	5	0	25
Varto	10	6	4	0	0	0	10
Total	159	96	63	51	19	32	210

Table 2. Distribution of horses and donkeys in the study by age

Age/Species	Horse	Donkey	Total
0-5	28	9	37
6-10	56	15	71
>10	75	27	102
Total	159	51	210

was intraperitoneally administered 0.2 ml of diluted liquid containing tachyzoites. The obtained exudate was diluted with NaCl and homogenized, and when examined under a light microscope by adding activator serum, it was passaged to contain 25-30 tachyzoites. The obtained sera were then diluted in 1/16, 1/64, 1/128 ratios and the same amount of antigen (mixed with activator serum) was added and incubated for 50 minutes in a 37^0C water bath. After incubation, 0.025 ml of alkali methylene blue was added and mixed. 0.020 ml of it was taken and placed on the slide; applying a coverslip, it was examined at magnification under light microscope (10×40).The evaluation was made according to the staining pattern of the tachyzoites under the light microscope; if a tachyzoite was dyed more than 50%, the test was considered to be negative. If there was a tachyzoite that wasn't dyed more than 50%, the test was considered to be positive.

Statistical analysis

Statistical analysis of the data was made by SPSS 20.0 (SPSS Inc., Chicago, IL, USA) program to determine the seropositivity and seronegativity significance levels between the species, gender and age groups using the *Chi-square test* (X^2). Statistical significance in this study was defined as $P<0.05$.

RESULTS

Serological findings of horses and donkeys

While seropositivity were detected in 68 (42.77%) of 159 horses, 91 (57.23%) of them were seronegative. At the same time, while seropositivity were determined in 47 (92.16%) of 51 donkeys, seronegative were 4 (7.84%) of them. As a result, 115 (54.76%) equidae were found to be seropositive in total, and 95 (45.23%) found to be seronegative (Table 3).

Distribution of *T. gondii* seropositivity and antibody titers detected by SFDT in horses by gender and age

T. gondii antibodies were determined in 68 (42.77%) of the horse sera examined by SFDT. Of the seropositive sera; 66 (41.50%) yielded positivity at a titer of 1/16and 2 (1.25%) at 1/64. *T. gondii* seropositivity was determined as 47.92% (46/96) in females and 32.92% (22/63) in males. In addition, *T. gondii* seropositivity was detected as 46.67% (35/75) in those older than 10 years, as 42.86% (24/56) in those aged 5-10 years, and 32.14% (9/28) in 0-5 age group. However, there was no statistically significant difference between both gender and age groups (P>0.05) (Table 4).

Table 3. Serological findings of horses and donkeys

Species	Number of sera	Number and rate of positivity (%)	Number and rate of negativity (%)	*P value*	Antibody titer	
					1/16	1/64
Horse	159	68 (42.77%)	91 (57.23%)	0.001	66	2
Donkey	51	47 (92.16%)	4 (7.84%)		38	9
Total	210	115 (54.76%)	95 (11.58%)		104	11

Table 4. Distribution of *T. gondii* seropositivity and antibody titers detected by SFDT in horses by gender and age

Factor		Number of sera	Number and rate of positivity (%)	Number and rate of negativity (%)	*P value*	Antibody titer	
						1/16	1/64
Gender	**Female**	96	46 (47.92%)	50 (52.08%)	0.105	45	1
	Male	63	22 (% 32.92)	41 (65.08%)		21	1
Age	**0-5**	28	9 (32.14%)	19 (67.86%)	0.897	7	2
	5-10	56	24 (42.86%)	32 (57.14%)		24	0
	>10	75	35 (46.67%)	40 (53.33%)		35	0
	Total	159	68 (42.77%)	91 (57.23%)		66	2

Table 5. Distribution of *T. gondii* seropositivity and antibody titers detected by SFDT in donkeys by gender and age

Factor		Number of sera	Number and rate of positivity (%)	Number and rate of negativity (%)	P value	Antibody titer		
						1/16	1/64	1/128
Gender	**Female**	19	17 (% 89.47)	2 (% 10.53)	0.547	13	4	0
	Male	32	30 (% 93.75)	2 (% 6.25)		25	4	1
Age	**0-5**	9	7 (% 77.78)	2 (% 22.22)	0.198	5	2	0
	5-10	15	14 (% 93.33)	1 (% 66.67)		11	2	1
	>10	27	26 (% 96.30)	1 (% 3.70)		22	4	0
	Total	51	47 (% 92.16)	4 (% 7.84)		38	8	1

Distribution of *T. gondii* seropositivity and antibody titers detected by SFDT in donkeys by gender and age

T. gondii antibodies were determined in 47 (92.16%) of donkey sera examined by SFDT. Of the seropositive sera, 38 (74.51%) yielded positivity at a titer of 1/16, 8 (15.69%) at 1/64, and 1 (1.97%) at 1/128.*T. gondii* seropositivity was determined as 89.47% (17/19) in females and 93.75% (30/32) in males. In addition, *T. gondii* seropositivity was detected as 96.30% (26/27) in those older than 10 years, 93.33% (14/15) in those aged 5-10 years, and 77.78% (7/9) in 0-5 age group. However, there was no statistically significant difference between both gender and age groups (P>0.05) (Table 5).

DISCUSSION

The first study with horses in Turkey was conducted on 154 horses by SFDT about 50 years ago and seropositivity at a rate of 14.3% was detected (Dubey, 1998).It is reported that, under natural conditions, the prevalence of toxoplasmosis in horses ranges from 0% to 90% worldwide (Akkan et al., 2001).In addition, it is stated that the seroprevalence of *T.gondii* in donkeys worldwide varies between 11% and 62% (Machacova et al., 2014).Many factors are held responsible for this wide range of seropositivity, such as sensitivity and specificity of the serological test used, age of animals, climate, breeding and care standards, hygiene of shelters, and the number of samples taken (Pomares et al., 2011; Machacova et al., 2014).In the present study, the areas where horses and donkeys live, shelter conditions, shelter hygiene and contact with stray animals in Muş province support these suggestion.

Toxoplasma gondii causes subclinical infections in equidae (horses and donkeys). Therefore, the diagnosis of the infection is performed using various serological tests to detect *T. gondii* antibodies. A number of serological tests to detect antibodies to *T. gondii* have been thoroughly studied in various hosts (Dubey and Beattie, 1988).Although the requirement for the use of live parasites means that the SFDT is not commonly used, it remains the gold standard in many hosts.We therefore selected it for our study.Using the test, we found that the overall seroprevalence of toxoplasmosis 42.77% (68/159) in horses and % 92.16 (47/51) in donkeys in the province of Muş. Also, a statistical difference was observed between species (P<0.001) (Table 3).

When studies on Toxoplasmosis seroprevalence in horses are reviewed worldwide, varying levels of seropositivity have been reported. By different serological methods (ELISA, IFAT and MAT), seropositivities have been determined between 11.59%-22.7% in Brazil (Ribeiro et al., 2016; Almeida et al., 2017; Magalhães et al., 2017) %37.8-39% in Romania (Paştiu et al., 2015), 26% in Algeria (Mohamed-Cherif et al., 2015), 17.7% in Tunisia (Boughattas et al., 2011) and 14% in Iran (Razmi et al., 2016). The presented study, seropositivity (42.77%) detected in horses by was found to be higher than the seropositivity rates obtained by the study conducted in many countries (Table 3). The differences in the results from various studies worldwide on *T. gondii* seroprevalence in horses can be attributed to the factors such as types of serological tests employed, age of horses, location of the studies, the intended use of the animals, the number of final cat hosts and the level of contact thatthe horses have with these cats (Miller et al., 1972; Machacova et al., 2014; Paştiuet al., 2015).In our study, the high seropositivity in horses was attributed to related reasons.

When studies on toxoplasmosis seroprevalence in donkeys are reviewed worldwide, various levels of seropositivity have been reported. By different serological methods (ELISA, IFAT, LAT, MAT and PCR), seropositivities have been determined between 45% and 65% in Egypt (El-Ghaysh et al., 1998, Haridy et al., 2010), 5-8% in Italy (Machacova et al., 2014), 25.6% in USA (Dubey et al., 2014), 34% in Spain (García-Bocanegra et al., 2012), and 6.29% in China (Zhang et al., 2017). Also, two separate studies conducted in Egypt and China has linked the high rate of seropositivity to free rearing style of donkeys and to greater contact with cats (El-Ghaysh et al., 1998; Zhang et al., 2017). In addition, Machacova et al. (2014) have attributed the differences in seroprevalence rates to the number of donkeys subjected to test.In the present study, the seropositivity rate was determined as 92.16%, which was above the world average (Table 3). In addition, it was observed that high seropositivity was compatible with the related studies. That is, the free breeding of the donkeys tested explains the high rate of seropositivity.

When studies related to toxoplasmosis seroprevalence in horses in Turkeyare reviewed, various levels of seropositivity have been reported with different serological methods. By different serological methods (ELISA, IHAT, and SFDT), seropositivities have been determined between 2%-63.09% in Ankara (Babur et al., 1997; Babur et al., 1998; Güçlü et al., 2007; Gazyağcı et al., 2011), 10.44% in Kayseri (İnci et al., 2002), 7.2% in Niğde (Karatepe et al., 2010), 6.4% in Malatya (Aktaş et al., 1999), 1.80 % and 20.6%

in Kars (Aslantaş et al., 2001; Akça et al., 2004), % 1.74 in Van (Akkan et al., 2001), 13.5% and 28.4% in Hakkari (Göz et al., 2007), 6.35% in various provinces of Southeastern Anatolia (Diyarbakır, Gaziantep and Şanlıurfa) (Özkan et al., 2002) and 46.3% in the samples collected from various provinces of Turkey (Adana, Bursa, Gaziantep, İstanbul, İzmir and Konya) (Zhou et al., 2016). In the presented study, it is observed that the seropositivity rate is parallel to the seropositivity rates determined in other studies (Table 3).

When studies on toxoplasmosis seroprevalence in donkeys are reviewed in Turkey, various levels of seropositivity have been determined. İnci et al. (2002) have reported that seropositivity in 14 of the 33 donkeys (% 42.42) in Kayseri, and Balkaya et al. (2011) determined seropositivity in 57 of the 92 (62%) by SFDT in Erzurum.In the presented study, it was noted that the seropositivity was higher than the seropositivity rates determined in other studies (Table 3).The number of animals sampled can be considered as the reason for this difference.

Considering the relationship between toxoplasmosis and age in horses, Boughattas et al. (2011) have achieved a seropositivity rate of 21.27% in those over the age of 10; whereas Villa et al. (2018) reported a high rate of seropositivity in horses over 15 years old. Klun et al. (2017) have indicated that age was not statistically significant and they attributed the low rate of seropositivity to the young study population. In Turkey, Göz et al. (2007) stated that seroprevalence is higher in horses within the age range of 0-2 in Hakkari province. Karatepe et al. (2010) have reported that there was no statistical difference between the groups, although they detected seropositivity at a rate of 7.40% in 1-10 age group and 6.81% in 11-20 age group, in Niğde. In the present study, high levels of seropositivity were detected in horses over 10 years of age. However, as in other studies, no statistically significant difference was found between age groups (Table 4).

Looking at the relationship between toxoplasmosis and age in donkeys, Dubey et al. (2014) detected seropositivity in donkeys older than 30 months, while they did not detect seropositivity in those younger than 30 months. Machacova et al. (2014) stated that the positivity was higher in the animals in the elderly group, in their study by the LAT and IFAT. Balkaya et al. (2011), despite detecting seropositivity at rates of 38.6% in the age group 0-3, 50.9% at 4-6, and 19.3% at over the age of 7 in Erzurum Province, have not reported a statistical difference between age groups. In the presented study, high levels of seropositivity were detected in donkeys over 10 years of age. However, as in other studies, no statistically significant difference was found between age groups (Table 5).

When *T. gondii* seroprevalence in horses were evaluated with regards to gender groups, Haridy et al. (2009) have determined in Egypt, higher seropositivity in females (50%) than in males (22.2%), and inferred that females were more sensitive to the agent.Göz et al. (2007) reported a higher rate of seropositivity in females in Hakkari, while Güçlü et al. (2007) revealed a higher rate of seropositivity in males in Ankara. However, no statistical difference was found between the genders in both studies. In the present study, higher seropositivity was determined in females. However, as in other studies, no statistically significant difference was found between gender groups (Table 4).

When *T. gondii* seroprevalence in donkeys were evaluated with regards to gender groups, Haridy et al. (2010) in Egypt, Dubey et al. (2014) in USA and Machacova et al. (2014) in Italy have reported higher seropositivity rates in female donkeys.In addition, in Erzurum province of Turkey, a higher seropositivity rate has indicated in female donkeys, but no statistical difference was observed (Balkaya et al., 2011).On the other hand, in the present study, a higher seropositivity was found in male donkeys. However, as in other studies, no statistically significant difference was found between gender groups (Table 5).

In conclusion, Toxoplasmosis seroprevalence in horses and donkeys in Muş region of Turkey was determined as 42.77% in horses and as 92.16% in donkeys. The infection with zoonotic character has been ascertained to be critical for the public health and therefore should be taken in consideration. Also, it is necessary to conduct extensive research on the role of livestock in the epidemiology and transmission of Toxoplasmosis in Turkey.

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*This study is a summary of the first name author's master thesis.

CONFLICT OF INTEREST

None reported.

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