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Investigation of Seroprevalence of *Toxoplasma gondii* in Sheep and Goats in Siirt Province in Turkey

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Abstract

This study was carried out to determine the seroprevalence of toxoplasmosis in sheep and goats in Siirt province. Blood samples were collected from a total of 450 female animals, consisting of 200 goats and 250 sheep in different part of Siirt province. Samples were centrifuged at 3000 rpm for 10 minutes and stored at -20 °C until use. The study was carried out by ELISA method using commercial test kit (*Toxoplasma gondii* Antibody Test Kit, IDEXX). According to the results of this study 28 of the 450 animals (6.22%) were positive, 393 (87.33%) were negative, 17 (3.78%) were suspicious, while 12 (2.67%) were weak positive. As the result of the analyzes performed using the chi-square test a significant difference was found between the results and the species ($p<005$), and no significant difference between the results and the ages group of the animals was observed ($p>0.05$). As a result, the presence of *T. gondii* in sheep and goats in Siirt region was determined for the first time by this study. Since this study provides information about the current situation of *T. gondii* infection in Siirt region, it is thought that it will contribute to taking necessary measures in terms of public health and disease control practices.

Key Words: ELISA, goat-sheep, Siirt, toxoplasmosis, Turkey

Siirt İli Koyun ve Keçilerinde *Toxoplasma gondii* Seroprevalansının Araştırılması

Öz

Bu çalışma Siirt ilinde yetiştirilen koyun ve keçilerde toksoplazmozis seroprevalansını belirlemek amacıyla gerçekleştirildi. Siirt ilinin farklı çalışma merkezlerinden 200 keçi ve 250 koyun olmak üzere 450 dişi hayvandan kan örneği alındı. Alınan örnekler 3000 devirde 10 dakika santrifüj edildi ve kullanılıncaya kadar -20 °C de saklandı. Çalışma ticari test kiti (*Toxoplasma gondii* Antibody Test Kit, IDEXX) kullanılarak ELISA yöntemiyle gerçekleştirildi. Çalışma sonucunda 450 hayvanın 28 (%6.22)'i pozitif, 393 (%87.33)'ü negatif, 17 (%3.78)'si şüpheli ve 12 (%2.67)'si zayıf pozitif tespit edildi. Ki-kare testi kullanılarak yapılan analizlerde, sonuçlar ile tür arasında anlamlı bir fark bulunurken ($p<005$), sonuçlar ile yaş grupları arasında anlamlı bir fark tespit edilmemiştir ($p>0.05$). Sonuç olarak Siirt yöresindeki koyun ve keçilerde *T. gondii* varlığı ilk kez bu çalışma ile belirlenmiştir. Bu çalışma Siirt bölgesindeki *T. gondii* enfeksiyonunun mevcut durumu hakkında bilgi verdiği için halk sağlığı ve hastalık kontrol uygulamaları açısından gerekli önlemlerin alınmasına katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: ELISA, koyun-keçi, Siirt, toksoplazmozis, Türkiye

INTRODUCTION

Toxoplasmosis is a zoonotic parasitic disease commonly present all throughout the world, caused by an obligate intracellular protozoon, *Toxoplasma gondii* (1-3). *T. gondii* causes disease in poultry, reptiles, all mammals in the world, including humans (3, 4). The definitive hosts are animals connected to the family of cats and feline, while the intermediate host is often birds or mammals, and can be a human as well (3, 5). The presence of oocytes in water and nutrients contaminated with infected cat feces is reported to be of primary importance in the spread of the infection to the sheep, along with the potential infections through the congenital path and infected sperms (6). Infected sheep are reported to have chronic infection throughout their lives, and undercooked sheep meat was reported as a significant source of infection for humans (2). Cysts excreted through cat feces and the tissue cysts of the infected animals act as the source of the infection (6). Humans and pets living in areas where stray cats are high in numbers are at the risk of infection (1). Infected cats can excrete millions of oocysts with their feces and cause large-scale environmental contaminations, which can create an important source of infection during the pasturing of herbivorous animals (7, 8). Toxoplasmosis was reported as the most important parasitic abortion and neonatal death cause in sheep and goat (9). While the disease often has a sub-clinic course in sheep and goat, it can still cause pneumonia, enteritis, neurological dysfunctions, encephalitis, low birth weight, abortus, and death (10-13).

Because the disease has a subclinical or asymptomatic course, serologic methods such as Sabin-Feldman dye test (SFDT), Indirect fluorescent antibody test (IFAT), Latex agglutination test (LAT), Enzyme- linked immunosorbent assay (ELISA) (4, 10, 14), Modified agglutination test (MAT), Direct agglutination test (DT), Complement fixation test (CF) and Indirect hemagglutination assay (IHA) are used in the diagnosis of toxoplasmosis (14). The Sabin-Feldman dye test is accepted as the reference test in the diagnosis of toxoplasmosis (5, 15, 16). That being said, the fact that this test requires experienced researchers and a high-security laboratory, makes the more practical ELISA tests the preferred diagnosis method (9, 15, 16).

This study was carried out to determine the seroprevalence of toxoplasmosis in sheep and goats in Siirt province using the ELISA method.

MATERIALS AND METHODS

The Study Area

Siirt province (Fig. 1) is located in the Southeastern Anatolia, which is a semi-arid climate region. The average highest and lowest temperatures are 36.9 °C and 18.9 °C in summer, and 8.7 °C and -0.5 °C in winter. Water shortages and droughts are frequent during the summer (17).

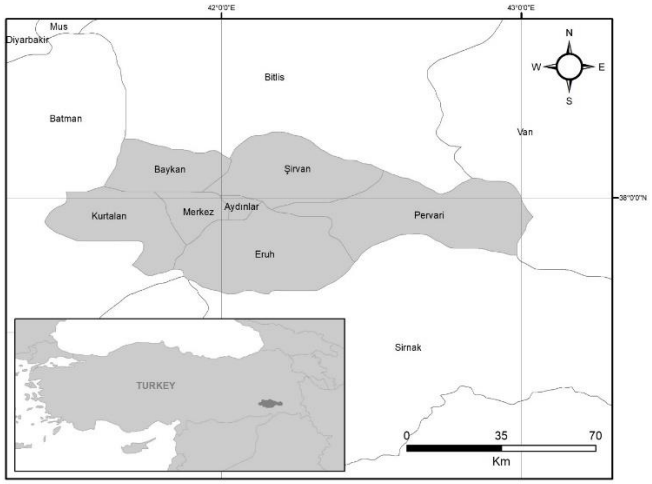

Figure 1. The map of Siirt province, in which the study was performed

Animal Material

The animal material of the study consists of a total of 450 female small ruminants consisting of 250 sheep and 200 goats with their ages ranging between 1 and 5 years, all raised in the Siirt province and selected randomly.

Sample Collection and Preparation

Blood samples that were taken from the jugular vein of the animals were centrifuged at 3000 rpm for 10 minutes, and their sera were obtained. The sera were stored at -20°C until further analysis.

Serological Examination

The ELISA tests of the study were performed in the Siirt University Science and Technology Application and Research Center. The commercial kit (*T. gondii* Antibody Test Kit, IDEXX Laboratories, Inc., Westbrook, Maine, USA) was used for the detection of anti-*Toxoplasma gondii* antibodies. The test was performed according to the procedure described by the manufacturer and the results were assessed at a wavelength of 450 nm using an ELISA microplate reader (Thermo Scientific Multiskan Go, Thermo Well Wash). The following equation was used:

$$Value\% = \frac{OD\ (Sample) - OD\ (Negative\ Control\ Mean)}{OD\ (Positive\ Control\ Mean) - OD\ (Negative\ Control\ Mean)} \times 100$$

If the value is equal to or greater than 100, then it is interpreted as positive. If the value is equal to or greater than 30 and less than 100, then it is interpreted as weak positive. If the value is equal to or greater than 20 and less than 30, then it is interpreted as suspect. If the value is less than 20 then it is interpreted as negative.

Statistical Analysis

The data obtained from the study were analyzed by using SPSS program pack. The difference between group variables was determined by chi-square analysis. A value of $p<0.05$ obtained through the analysis was considered statistically significant.

Ethical Approval

Ethical approval for this study was obtained from the Dicle University Local Ethics Committee for Animal Experiments. (Protocol number: 2015/16).

RESULTS

Of the 450 animals examined using the ELISA method, 28 (6.22%) were positive, 393 (87.33%) were negative, 17 (3.78%) were suspicious and 12 (2.67%) were weak positive. When the species were analyzed separately, 11 (5.50%) of 200 goats were found to be positive, while 183 (91.50%) were negative, 5 (2.50%) were suspicious, and 1 (0.50%) was weak positive. Of the 250 sheep samples examined, 17 (6.80%) were identified as positive, 210 (84%) were negative, 12 (4.80%) were suspicious and 11 (4.40%) were weak positive. In goats grouped according to their age, 9 (7.56%) seropositivity was detected in those equal or less than 3 years old and 2 (2.47%) seropositivity was detected in those greater than 3 years. In sheep, 11 (7.91%) seropositivity was detected in those equal or less than 3 years old and 6 (5.41%) seropositivity was detected in those greater than 3 years. When both types are evaluated together, 20 (7.75%) seropositivity was detected in those equal or less than 3 years old, and 8 (4.16%) seropositivity was detected in those greater than 3 years old. The result of the analysis using chi-square (x^2) test revealed that there was a significant difference between the results (weak positive and negative) and the species ($p<0.05$), while there was no significant difference between the results and the ages of the animals ($p>0.05$) (Table 1).

Table 1. Distribution of results by species and age group

	Positive		Negative		Suspicious		Weak positive		P
	n	%	n	%	n	%	n	%	
Species									
Sheep	17	6.8	210[a]	84	12	4.8	11[a]	4.4	0.031
Goat	11	5.5	183[b]	91.5	5	2.5	1[b]	0.5	
Age Group									
Sheep									
≤3	11	7.91	114	82.01	9	6.47	5	3.60	0,391
>3	6	5.41	96	86.49	3	2.70	6	5.41	
Goat									
≤3	9	7.56	107	89.92	2	1.68	1	0.84	0,281
>3	2	2.47	76	93.83	3	3.70	0	0.00	
Total									
≤3	20	7.8	221	85.7	11	4.3	6	2.3	0.375
>3	8	4.20	172	89.60	6	3.10	6	3.10	

It is written the values with different superscript letters in a column are significantly different ($p<0.05$).

DISCUSSION AND CONCLUSION

Parasitic diseases usually have a latent infection course and cause significant economic losses. Toxoplasmosis is a parasitic zoonotic disease and is very important in terms of public health, and people who have cats as pets, who work in animal husbandry, as butchers, or veterinary physicians are in the risk group (5, 10, 11).

The prevalence of toxoplasmosis in sheep in the world has been reported as 28.5% in Italy (18), 12% in Chile (19), 24.50-35% in Iran (20, 21), 6.7% in Nigeria (22), 18.75% in Brazil (23), 11.2% in Pakistan (24), 52.6% in Ethiopia (25), 30% in Mexico (26) and 52.2% in Saudi Arabia (27). The prevalence of *T. gondii* in goats in Europe was found to be between 5-91% in studies on the prevalence of toxoplasmosis in goats (28), while it was found as 21-82% in the Czech Republic (29, 30), 4.6% in Nigeria (22), 28.93% in Brazil (23), 25.4% in Pakistan (24), 19.25-30% in Iran (20, 21), 51.7% in Saudi Arabia (27), 24% in Ethiopia (25), and 44% in Mexico (26).

Various studies were performed in different regions of Turkey to determine the prevalence of *T. gondii*. In a study performed in Elazig province to determine the *T. gondii* antibodies in pregnant and aborted animals, the researchers have found that 72 out of 154 sheep (46.8%) had *T. gondii* seropositive sera with SFDT (31). In a study performed in the province of Yozgat on a total of 152 sheep above 1 year of age, the serum samples were investigated with SFDT in terms of *T. gondii* antibodies, and 69 (45.4%) of the sheep were identified as seropositive (4). Karatepe ve ark. (2001) obtained blood samples from a total of 108 sheep above 1 year of age in the Gümüşhaciköy district of Amasya province, and tested the serums with SFDT for *T. gondii* antibodies. The researcher found that 72 out of 108 sheep (66.6%) were seropositive.

Another study investigated 99 sheep slaughtered in Mersin province meat and fish authority with the with SFDT, and found that 48 (48.4%) of the animals were seropositive (33). The serum samples of 172 sheep raised in the Afyon province were controlled with SFDT for *T. gondii* antibodies, and 94 (54.65%) of them were found to be seropositive (2). In a study which investigated the *T. gondii* prevalence in the blood samples of a total of 100 sheep with SFDT, the seropositivity was determined as 72% in Aydın region (14). A similar test study was performed to determine the *T. gondii* prevalence in a sample group of 63 sheep in Yalova province with with SFDT and LAT, and the seropositivity for the tests were reported as 66.66% and 65.08%, respectively (34).

Mor and Arslan (2007) have performed a study over the blood samples of a total of 460 sheep who tested the samples with ELISA method, and determined the *T. gondii* seroprevalence as 95.7% in the Kars region. A study investigated the *T.gondii* prevalence in sheep groups which had and hadn't aborted using IFAT test where a total of 184 sheep were tested, and 24 (13%) were found seropositive. In the 106 sheep which had aborted, 12 (11.3%) were seropositive, and in 78 sheep which hadn't aborted, 12 (15.4%) were seropositive with *T. gondii* antibodies in Karapınar district of Konya province (1).

In a study performed in the Şuhut district of Afyonkarahisar province over 186 sheep to determine the spread of toxoplasmosis, the serological evaluations have revealed that 184 out of 186 animals (98.92%) were seropositive with SFDT (6). In the Hatay region, blood samples obtained from 184 sheep and 184 goats were tested with ELISA method, and the seropositivity were determined as 53.8% and 35.9%, respectively (9). Blood samples from 180 sheep in the Nevşehir region were collected and tested with ELISA method, and a seropositivity of 10% was determined (35). Similarly, the samples obtained from 230 sheep and 150 goats raised around Kars region were tested in another study with ELISA method for anti-*T.gondii* antibodies, and 25 sheep (10.87%) and 23 goats (15.33%) (10). Samples from a total of 234 sheep with different breed, age, and gender from Adana region were tested for *T.gondii* prevalence with SFDT

method, and the seropositivity was determined as 78.6% (34). Celik ve ark. (2018) performed a study in the Siirt province where they determined the *T.gondii* seroprevalence in 300 serum samples obtained from cattle with ELISA method, and found 53 (18%) were seropositive. Güler and Karatepe (2019) performed a study where they determined the anti-*T. gondii* antibodies in sheep slaughtered in Niğde province slaughterhouse, and found that of the 175 samples tested, 11 (6.28%) were seropositive with ELISA method.

In the present study, the *T. gondii* seroprevalence in sheep and goats of Siirt province were determined as 6.80% and 5.50%, respectively. This relatively low seropositivity ratio is in line with the results reported by Güler and Karatepe (2019). The finding of this study that the seropositivity of the sheep is higher compared to the seropositivity of the goats is concurrent with the findings of the study of Muz ve ark. (2013)**,** while the finding that no statistically significant differences exist between different age groups is in line with the findings of Çakmak and Karatepe (2017) and Ekşi ve ark. (2018).

The finding that the seropositivity of goats determined as a result of this study (5.5%) is lower than that of the sheep (6.80%), and that of the seropositivity determined in the Siirt province (18%) by Celik ve ark. (2018), might be due to the fact that sheep and cows usually feed off from the ground, while the goats feed from small bushes and oaks. The relatively large differences between the studies performed in Turkey, and similarly throughout the world, might be due to differences of geographical conditions, diagnostic methods used, design patterns of the studies, or variations regarding the animal species, breed, and numbers investigated, or the prevalence of feline in the study region.

The fact that this is a zoonotic disease poses a serious risk for humans. The disease is commonly present throughout the world, and the spread of toxoplasmosis in human populations in Turkey varies between 17.6% and 76.6% (15). The infection can spread to humans through accidental contact with oocytes dispersed through feline feces, consumption of raw or undercooked meats that contain bradyzoite forms, or through the transplacental path that transmits the disease from the mother to the fetus (15, 16). The disease has a subclinical course in individuals with strong immune systems, while it can become fatal for those with immune deficiencies (16). Congenital infections can result in preterm delivery, intraocular infections, blindness, microcephaly, mental retardation, hydrocephaly, and hepatosplenomegaly (39). As such, studies suggest that in regions where the disease is commonly encountered, women who are pregnant or are in the reproductive age group should frequently be surveyed for it (16).

Control over stray cats forms an important part of the efforts to prevent and control the spread of toxoplasmosis, as they act as the definitive host for the parasite. Farm animals and their feed should be isolated from any potential cat feces. Meats and meat products should not be consumed raw or undercooked, and vegetables and fruits should only be consumed once they are thoroughly washed. Women should have a pre-pregnancy test to determine their health status, and negative women should take the necessary precautions to avoid the disease during pregnancy. The presence of *T. gondii* in sheep and goats in Siirt region was determined for the first time by this study. Since this study provides information about the current situation of *T. gondii* infection in Siirt, it is thought that it will contribute to taking necessary measures in terms of public health and disease control practices.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests.

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