Seroprevalence of *Toxoplasma gondii* in Sheep from Nevşehir Province in Turkey

Nevşehir Yöresi Koyunlarında *Toxoplasma gondii*'nin Seroprevalansı

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ABSTRACT

Objective: The goal of this study was to investigate the seroprevalence of *Toxoplasma gondii* (*T. gondii*) in sheep from Nevşehir Province in Turkey.
Methods: Blood samples were taken from 180 sheep aged between 1 and 7 years, which were randomly selected from seven different study sites in Nevşehir Province. The enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *T. gondii* was performed on all the serum samples.
Results: Eighteen (10%) serum samples were found to be seropositive for *T. gondii* antibodies. The highest seropositivity rate (11.53%) was found in sheep aged between 1 and 2 years, whereas the lowest seropositivity rate (8.51%) was found in sheep aged between 5 and 7 years old. Eighteen of the 162 ewes (11.1%) were found to be seropositive, whereas none of the 18 tested rams were seropositive. In addition, considering the study site location, the highest seropositivity rate was in Avanos (32%), whereas the lowest seropositivity rate was in Kozakli (2.9%). The *T. gondii* seropositivity rates were statistically insignificant with regard to age groups and gender ($p>0.05$), whereas they were statistically significant ($p<0.05$) with regard to study centers.
Conclusion: This is the first serological report on toxoplasmosis in sheep from Nevşehir Province in Turkey.
Keywords: *Toxoplasma gondii*, sheep, Nevşehir, seroprevalence, ELISA, Turkey
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ÖZ

Amaç: Bu çalışma, İç Anadolu Bölgesinde Nevşehir yöresinde bulunan koyunlarda *Toxoplasma gondii (T. gondii)*'nin seroprevalansının araştırılması amacı ile yapılmıştır.
Yöntemler: Çalışmanın materyali olarak Nevşehir ilinde 7 farklı çalışma merkezine ait (Gülşehir, Nevşehir Merkez, Kozaklı, Acıgöl, Avanos, Hacıbektaş, Derinkuyu) 1-7 yaş arasında toplam 180 koyun rastgele seçilerek kan örnekleri alınmıştır. Alınan kan örneklerinin serumları çıkarılmış *T. gondii* antikorları yönünden ELISA testi ile incelenmiştir.
Bulgular: Nevşehir yöresinde ELISA testi ile incelenen toplam 180 koyunun 18 (%10)'inin *T. gondii* antikorları yönünden seropozitif olduğu tespit edilmiştir. Bunun yanında en yüksek seropozitiflik %11,53 oranı ile 1-2 yaşındaki koyunlarda tespit edilirken en az seropozitiflik oranı ise %8,51 ile 5-7 yaş arası koyunlarda belirlenmiştir. Cinsiyetlere göre 162 koyunun 18 (%11,1)'inde seropozitiflik tespit edilmiş, buna karşılık 18 koçta seropozitiflik saptanmamıştır. Ayrıca çalışma merkezleri açısından koyunlarda en yüksek seropozitiflik %32 oranı ile Avanos'da, en düşük seropozitiflik ise %2,9 oranı ile Kozaklı'da tespit edilmiştir. Elde edilen seropozitiflik oranlarında; koyunların yaş grupları ve cinsiyetlerinin istatistiksel olarak önemli ($p>0,05$) olmadığı, çalışma merkezlerinin ise istatistiksel olarak önemli olduğu saptanmıştır ($p<0,05$).
Sonuç: Nevşehir yöresinde koyunlarda *Toxoplasma gondii*'nin varlığı ilk kez bu çalışma ile belirlenmiştir.
Anahtar Kelimeler: *Toxoplasma gondii*, koyun, Nevşehir, seroprevalans, ELISA, Türkiye
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INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the intracellular protozoan parasite *T. gondii*, and it may affect all mammals, humans, and birds (1-4). The definitive hosts for the *T. gondii* are cats and other felines, whereas the intermediate hosts are all the birds and mammals, including humans (2, 4-6).

Toxoplasmosis in sheep and other animals is subclinical. In some acute cases, toxoplasmosis may cause symptoms such as an increased body temperature, loss of appetite, diarrhea, lack of energy, and breathing problems. Those symptoms are generally not typical of the disease; therefore, the diagnosis of toxoplasmosis in sheep only by observing the symptoms is difficult. As with other organisms, the diagnosis of toxoplasmosis in living sheep can be achieved by various serological methods (1).

The Sabin-Feldman dye test (SFDT), indirect fluorescent antibody test (IFAT), indirect hemagglutination test (IHA), complement fixation test (CFT), and enzyme-linked immunosorbent assay (ELISA) are some of the various tests available to diagnose toxoplasmosis (1, 5, 6).

Previous studies conducted in Turkey indicate that the average prevalence of toxoplasmosis in sheep is 40%, with regional variations ranging between 2.8% and 95.7% (7-15).

The aim of this study was to determine the seroprevalence of *T. gondii* infection in sheep from Nevşehir Province in Turkey.

METHODS

Study Area

The material used in this study was the blood serum samples taken from randomly selected 180 sheep aged 1 to 7 years. This study included sheep from Nevşehir Province, located in the Central Anatolia Region, Turkey (with an altitude of 1260 m, 38° 37′ N longitude-34°43′ E latitude), where toxoplasmosis has not been previously detected. In the present study, Gulsehir, Nevşehir Central, Kozakli, Acigol, Avanos, Hacibektas, and Derinkuyu districts were chosen as study sites in Nevşehir Province. In Nevşehir, the annual average precipitation is 429.4 kg/m^3, the average temperature 10.6°C, and the average relative humidity 60.6%.

Blood samples were collected in a sterile tube from the punctured jugular vein of sheep. Serum samples were obtained by centrifugation at room temperature (25°C), at 4 000 rpm for 10 minutes, and were stored at −20°C until analyzed. To detect the *T. gondii* antibodies, CHEKIT-Toxotest ELISA Test Kit (IDEXX, AG, Switzerland) was used in the study. The ELISA test was performed according to the instructions listed on the commercial kit.

Reading and Calculation of Results

The test was performed according to the procedure described by the manufacturer and the results were assessed at a wavelength of 450nm using an ELISA microplate reader (MR-96A). The following equation was used:

$$\text{Value\%} = \frac{\text{O.D. sample} - \text{O.D. negative}}{\text{O.D. positive} - \text{O.D. negative}} \times 100$$

If the test sample's percentage value was greater than or equal to 100%, the test was considered to be positive. If the result was greater than or equal to 30% or less than 100%, the test was weakly positive. If the result was greater than or equal to 20% and less than 30%, the result was suspicious. Finally, if the result was less than 20%, the test was considered to be negative.

Statistical Analysis

The chi-squared test was applied to compare the rate of seropositivity between age groups, gender, and study sites. Statistical significance in this study was defined as $p<0.05$.

RESULTS

Out of the 180 sheep aged between 1 and 7 years from seven different sites in Nevşehir Province, the ELISA test showed that 18 sheep (10.0%) had anti-*T. gondii* antibodies.

According to Table 1, the highest seropositivity rate of 11.53% was detected in 1- to 2-year-old sheep, whereas the lowest seropositivity rate of 8.51% was detected in 5- to 7-year-old sheep. As a result, the seropositivity rate among the seropositive animals with regard to the age groups of the sheep was not statistically significant ($p>0.05$).

Table 1. *Toxoplasma gondii* seropositivity rate by ELISA in sheep with regard to age groups

Age Groups	Animals Examined	Positive Animals	Seropositivity Rate (%)
1–2	78	9	11.53
3–4	55	5	9.09
5–7	47	4	8.51
Total	180	18	10.0

Table 2. *Toxoplasma gondii* seropositivity rate with regard to the study centers

Study Site	Month	Animals Examined	Positive Animals	Seropositivity Rate (%)
Gulsehir	February	38	2	5.3 [b]
Centre	March	23	2	8.7 [b]
Kozakli	April	34	1	2.9 [b]
Acigol	May	30	2	6.7 [b]
Avanos	June	25	8	32.0 [a]
Hacibektas	July	11	1	9.1 [b]
Derinkuyu	August	19	2	10.5 [b]
TOTAL		180	18	10.0

[a,b]The differences between a and b in the column are significant ($p<0.05$).

Table 2 shows the seropositivity rates in sheep according to the study sites. As the table indicates, the sheep in Avanos had the highest seropositivity rate (32.0%), whereas the lowest seropositivity rate was in Kozakli (2.9%). As a result, the seropositivity rate of *T. gondii* among the seropositive animals with regard to the study sites was determined to be statistically significant ($p<0.05$). In addition, the presence of cats was confirmed at all study sites.

With regard to the gender groups, out of 162 ewes, 18 (11.1%) were found to be seropositive, whereas no seropositivity was detected in the 18 rams tested. Seropositivity rates of the two gender groups were compared; the differences between the seropositive animals were not statistically significant ($p>0.05$). Out of the 8 sheep that miscarried, none were found to be seropositive.

DISCUSSION

In Turkey, the seroprevalence of toxoplasmosis in sheep was 20%–39.28% as indicated by CFT (8, 13), 36% by ToxHAtest commercial kit (16), 14.66%–37% by LAT (9, 17), 22.5% in gravid ewes, 30.97% in ewes by IHA (12, 18), 22%–95.7% by ELISA (14, 18), 28.04%–88.70% by SFDT (7, 9, 10, 13, 19-23), and 13%–72% by IFAT (9, 24, 25).

In our study, the seroprevalence of *T. gondii* was 10% in sheep from Nevşehir in Turkey. This seropositivity rate of 10% is clearly lower than the seropositivity rates observed in other studies carried out in Turkey. The differences in the results from various studies can be attributed to various factors, such as geographic differences, various types of the serological tests employed, the type and number of sheep examined, and the number and density of the definitive host, cats. Moreover, the rate of seroprevalence in this study was similar to the results found in Konya (24).

The seroprevalence of anti-*T. gondii* antibodies in sheep has been reported in several countries. The *T. gondii* seropositivity was found to be 7.4% by IHAT, 9.2% by IgG-ELISA, and 25.2% by IgM-ELISA in Australia (26); 65.5% by MAT in USA (27); 13.9% in small flocks of sheep and 28.5% big flocks of sheep by LAT and IFAT in Uruguay (28); 2.5% by LAT in Pakistan (29); 24.50% by LAT and IHAT in Iran (30); 12% by IHAT and 28% by IIFT in Chile (31); 18.75% by LAT in Brazil (32); 3.6% by MAT in North America (33); 13.8%–74.5% in Syria (34); 28.4% by IgG-IFAT; 9% by IgM-IFAT and 11.1% PCR in Italy (35); 27.6% by IgG-ELISA in Morocco (36); 26.5% by IFAT in Iran (37); and 19.88% by ELISA and LAT in Pakistan (38).

The seropositivity rate of 10% detected in this study is lower than the seropositivity rates reported in other studies from different parts of the world (27, 28, 30-32, 34, 36-38). On the other hand, the rates of 3.6% and 2.5% observed by Dubey and Foreyt in North America (33) and Zaki in Pakistan (29), respectively, are lower than the seropositivity rates observed in Nevşehir. These differences can be attributed to the differences in country of study, the type of the serological tests used, the differences in the sheep examined, and the number of the definitive hosts, cats. Moreover, the seropositivity rate of 10% observed in this study is similar to the rates found in Australia (9.2%) and Italy (9–11.1%) (26, 35).

In the present study, although we found no differences in the *T. gondii* seroprevalence rates with regard to mean age among sheep ($p>0.05$), *T. gondii* antibodies in the 1–2-years age group were more common than in the 5–7-years age group. The difference was probably due to the presence of maternal antibodies or latent infection. In addition, other external factors, such as farm management or feeding behavior, could account for this discrepancy. Likewise, among the seropositive animals, statistical significance with regard to gender was not observed ($p>0.05$). However, the *T. gondii* seropositivity prevalence rate among the seropositive animals with regard to the location of study centers was found to be statistically significant ($p<0.05$), where Avanos was identified as having the highest seropositivity rate (32.0%), and Kozaklı the lowest seropositivity rate (2.9%). The differences between the seropositivity rates in the study centers could be attributed to the breeding conditions, the population of the definitive host cats, and the level of contact those cats have with sheep feeders and waterers.

CONCLUSION

The study results clearly identify the presence of *T. gondii* in the sheep from Nevşehir Province in Turkey. Consequently, *T. gondii* should be considered a possible cause of miscarriage in sheep. Therefore, considering the fact that cats are the definitive hosts and one of the most important factors in the spread of the infection, they should be kept away from the sheep feeders and waterers, and pens and the pastures, thus preventing contamination through cat feces (1, 2, 5). To determine the effects of toxoplasmosis on sheep breeding in Nevşehir, a larger study that would also investigate cats, the definitive hosts for *T. gondii,* is recommended.

Ethics Committee Approval: Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects".

Informed Consent: Not required in this study.

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