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Research Article

Determination of Seropositivity for *Toxoplasma gondii* in Sheep in Şanlıurfa Province

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Abstract: The aim of this study was to detect the seropositivity for *Toxoplasma gondii* in sheep in Şanlıurfa province using the Sabin-Feldman dye test. Serum samples collected from 300 sheep were tested for *T. gondii*. Serological examination revealed 167 (55.66%) sheep to be seropositive. Among these sera the numbers of seropositive samples at certain dilution steps were as follows: 65 at 1/16, 63 at 1/64, 27 at 1/256 and 12 at 1/1024. There was no significant correlation between seropositivity and the factors age, sex and breed. In conclusion, detection of a 34% seropositivity rate in 1/64 and higher dilutions indicates the clinical course of toxoplasmosis in the region.

Key Words: *Toxoplasma gondii*, sheep, Sabin-Feldman dye test

Şanlıurfa Yöresinde Koyunlarda *Toxoplasma gondii*'nin Seropozitiflik Oranının Belirlenmesi

Özet: Bu çalışmanın amacı, Şanlıurfa yöresindeki koyunlarda *Toxoplasma gondii*'nin seropozitiflik oranını Sabin Feldman boya testi ile belirlemekti. 300 koyundan toplanan serum örnekleri, *T. gondii* antikorları yönünden test edildi. Serolojik inceleme 167 (% 55,66) koyunun seropozitif olduğunu gösterdi. Bu serumlardan sulandırma basamağı 1/16'da 65, 1/64'de 63, 1/256'da 27 ve 1/1024'de 12 pozitif serum tespit edildi. Yaş, cinsiyet ve ırk faktörleri ile seropozitiflik oranı arasında önemli bir ilişki yoktu. Sonuç olarak, 1/64 ve daha yüksek dilusyonlarda % 34 oranında seropozitifliğin tespit edilmesi, bölgede toxoplasmosisin klinik olarak seyrettiğini göstermektedir.

Anahtar Sözcükler: *Toxoplasma gondii*, koyun, Sabin-Feldman boya testi

Introduction

A disease of zoonotic nature, toxoplasmosis has been reported to be widespread in animals throughout the world. Regarding the biology of *Toxoplasma gondii*, all mammals, including man, and birds are intermediate hosts, whereas Felidae act both as intermediate and definitive hosts. Sheep and goat meat are important infection sources for toxoplasmosis. Man acquires infection with *T. gondii* by consumption of raw and rare infected meat of intermediate hosts like sheep, goats and cattle, or by ingestion of sporulated oocysts via consumption of contaminated food and water. The infection is transmitted transplacentally as well (1-5).

Toxoplasmosis causes significant economic losses in sheep due to prenatal death and abortion. Similar to infection in other mammals, toxoplasmosis in sheep does not display specific clinical signs and follows a subclinical course. In some acute cases, atypical general disorders such as mild fever, inappetence, lethargy, dyspnea and occasionally diarrhoea may be observed (1,3-5). Due to difficulty in the diagnosis of toxoplasmosis according to clinical symptoms, serological tests are used for detection. The enzyme–linked immunosorbent assay (ELISA), complement fixation test (CFT), indirect haemagglutination test (IHAT), Sabin-Feldman (SF) dye test, indirect fluorescent antibody test (IFAT), latex

agglutination test (LAT) and modified agglutination test (MAT) are the most commonly used diagnostic tests. The SF test is the principal test for toxoplasmosis detection in both humans and animals (1,5,6).

The first study on *T. gondii* in sheep was carried out by Olafson and Monlux in 1942 (1). Data collected from serological studies in many countries throughout the world have shown different prevalence rates (0-100%) of toxoplasmosis in sheep (6-12). The first research on toxoplasmosis in sheep in Turkey was carried out in 1967 and the seropositivity rate was detected as 43.1% using the SF dye test (13). Data collected from studies carried out in different regions of Turkey in the following years have revealed a seropositivity range between 7.1% and 88.7%, differing according to the serological test used (14-20).

Due to the location of Şanlıurfa in south-eastern Turkey, the local custom of consuming traditional dishes prepared from raw meat is a threat to human health, and raw and rare sheep meat is a significant source of infection. Thus, the main objective of this study was to detect the seroprevalence of *T. gondii* infections in sheep bred in the Şanlıurfa region using the SF dye test and to discuss the possible risk factors.

Materials and Methods

This research was carried out on 300 sheep of different ages, sexes and breeds in Şanlıurfa and vicinity between July and October 2001. Sample processing and the SF test were performed according to Sabin and Feldman (21). Briefly, blood samples collected from sheep were centrifuged at 4000 rpm for 10 min at room temperature and the harvested sera were kept at –20 °C until used for testing. Prior to testing, serum samples were removed from the deep freeze and inactivated at 56 °C for 30 min for evaluation for the presence of anti-Toxoplasma antibodies using the SF dye test. The SF test was carried out at the Routine Toxoplasma Laboratory of the Ankara Refik Saydam Hygiene Directorate in accordance with the standard techniques using freshly prepared live antigens obtained from the ascites of Toxoplasma-infected mice and alkaline methylene blue staining. Titres of 1/16 and above were evaluated as positive. The chi-square test was used to assess the correlation between serum titres and the factors age, sex and breed (22).

Results

Results obtained regarding the sheep sera from the Şanlıurfa region and tested using the SF dye test are given in Tables 1 and 2.

Among the 300 sheep tested, 167 (55.66%) were detected to be seropositive. As seen in Table 1, among the 228 sheep tested belonging to the 0-1 age group, 133 (58.33%) were seropositive, whereas among the 72 sheep tested above the age of 1, 34 (47.22%) were seropositive. Among the 59 rams tested, 28 (47.45%) were seropositive, whereas among the 241 sheep tested, 139 (57.67%) were seropositive. Among the 208 Akkaraman breed sheep tested, 114 (54.80%) were seropositive, whereas among the 70 Ivesi breed sheep tested, 39 (55.71%) were seropositive. On the other hand, as seen in Table 2, among the 22 Morkaraman breed sheep tested, 14 (63.63%) were seropositive. The titres at which the 167 seropositive sheep were detected to be positive are as follows: 1/16 for 65, 1/64 for 63, 1/256 for 27 and 1/1024 for 12.

Table 1. *Toxoplasma gondii* seropositivity in Şanlıurfa province with regard to age, sex and breed.

	Age		Sex		Breed		
	0-1 age	1< age	Male	Female	Akkaraman	Ivesi	Morkaraman
The number of seronegative animals (%)	95 (41.66%)	38 (52.77%)	31 (52.55%)	102 (42.33%)	94 (45.20%)	31 (44.29%)	8 (36.37%)
The number of seropositive animals (%)	133 (58.34%)	34 (47.23%)	28 (47.45%)	139 (57.67%)	114 (54.80%)	39 (55.71%)	14 (63.63%)
Total	228	72	59	241	208	70	22

Table 2. Seropositivity titre rates with regard to age, sex and breed.

	Age		Sex		Breed		
Seropositivity Titres	0-1 age	1< age	Male	Female	Akkaraman	Ivesi	Morkaraman
1/16	48	17	9	56	44	16	5
1/64	49	14	13	50	41	17	5
1/256	24	3	5	22	21	4	2
1/1024	12	-	1	11	8	2	2
Total	133	34	28	139	114	39	14

The was no significant correlation between serum titres and age, sex or breed ($P > 0.05$).

Discussion

Many studies have been carried out in different countries throughout the world aimed at the detection of the prevalence of *T. gondii* in animals and to confirm its significance as an infection source for man by means of various serological tests including IHAT (8), LAT (9,10), MAT (11) and IFAT (7,12) as well as isolation procedures.

The SF dye test, CFT, IHAT, LAT, ELISA and IFAT have been used for detection of *T. gondii* antibodies in sheep in studies carried out in Turkey (13-19). The SF test used in this study is known to be the most reliable and sensitive method for the diagnosis of toxoplasmosis.

The results of the first research carried out by Ekmen (13) on 123 sheep revealed *T . gondii* seropositivity rates of 43.10% according to SF and 20% according to CF. In further studies, seropositivity was detected to be 38.00% by SF in 250 sheep serum samples collected from Ankara, Konya, Sivas and Trabzon by Weiland and Dalchow (14), and 36.50% in 148 sheep in Diyarbakır by Sarnıç (16). Altıntaş (15), reported 31.18% seropositivity according to the results of an SF test study carried out on 603 sheep in 6 cities in 1975. Altıntaş (17), reported the maximum and minimum seropositivity rates to be 55.19% and 25.32%, respectively, according to the results of an SF test study carried out on 2680 sheep kept at 17 state breeding farms in 1981.

According to studies carried out in Turkey aimed at the detection of the presence of *T. gondii* antibodies in sheep using tests other than SF, such as IHAT, LAT, ELISA and IFAT the highest seropositivity rate was measured by IFAT as 72.00% and the lowest rate by ELISA as 7.10% (18-20,23,24).

In further studies performed for the diagnosis of toxoplasmosis in sheep by the SF test, seropositivity rates were detected as follows: 69.80% in 414 sheep in the Ankara region by Babür et al. (24), 36.00% in 712 serum samples collected from 222 animals by Babür and Karaer (25), in Ankara, 88.70% in 62 sheep in the Çankırı region by Babür et al. (26), 40.59% and 33.00% in sheep in the Bala and Polatlı state breeding farms by Altıntaş et al. (27), 46.80% in 154 sheep in the Elazığ region by Aktaş et al. (28), 51.45% in 103 sheep in the Kars region by Aslantaş and Babür (29), 63.90% in 119 sheep in the Kırıkkale region by Yıldız et al. (30), 45.40% in 152 sheep in the Yozgat region by Babür et al. (31), and 66.66% in 108 sheep in the Amasya region by Karatepe et al. (32).

The course of toxoplasmosis in humans has also been traced in Turkey. According to the results of a study carried out between 1991 and 1995 in the Aegean region in 9410 patients of different ages, 4651 (49.40%) were seropositive for *T. gondii* and 2287 (21.40%) were determined to be pregnant. The pregnancy rate among seropositive patients was 55.00% and these women were reported to have experienced spontaneous abortions, stillbirth and birth of foetuses with abnormalities at rates of 50.00%, 52.00% and 55.00%, respectively (33).

In another recent study carried out by Aslan and Babür (34), in the province of Şanlıurfa, the seroprevalence of *T. gondii* among workers in sheep and cattle slaughterhouses was determined to be 48.83% using the SF test. Data obtained from the same study revealed seropositivity rates to be 42.74% in sheep and 49.13% in cattle. In the light of the high rate of

seropositivity in humans (approaching 50.00%) in the province of Şanlıurfa, it has been concluded that toxoplasmosis is a significant potential health hazard and titre measurement studies aimed at control should be continued on a regular basis every year. Comparison of the results of this study and the reality of 55.66% seropositivity confirms the tendency of the risk to increase over time.

When compared to other studies aimed at the diagnosis of toxoplasmosis by the SF test, the 55.66% seropositivity rate detected in 300 sheep sera collected from the Şanlıurfa region was lower than values detected in Çankırı (26), Kırıkkale (30) and Amasya (32), and similar to values measured in other studies. This difference was concluded to be due to the fact that the studies were carried out in different geographical regions. Altıntaş (15,17) reported seropositivity rates to differ among geographical regions, and factors including age, sex and breed to be insignificant regarding the incidence of toxoplasmosis. The statistical data of this study also confirmed the insignificance of age, sex and breed with regard to incidence.

Apart from causing significant economic losses in animal husbandry in Turkey, toxoplasmosis, through the consumption of rare or raw meat of infected animals by humans, leads to serious conditions such as abortions and stillbirth in pregnant women and malformations in babies (33).

In conclusion, the 55.66% seropositivity rate detected in sheep using the SF test and the 34.00% seropositivity rate detected in 1/64 and higher dilutions revealed the clinical course of toxoplasmosis in the region. Due to the economic losses observed in animal husbandry and the zoonotic characteristic of the infection, control measures should be taken against *T. gondii* and the consumption of raw or rare sheep meat by humans and especially cats, definitive host of the parasite, should be prevented.

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