

## The seroprevalence of *Toxoplasma gondii* in sheep detected by Sabin Feldman Dye Test in the region of Van, Turkey

Mehmet TÜTÜNCÜ<sup>1</sup> Hasan Altan AKKAN<sup>2</sup> Cahit BABUR<sup>3</sup> Erol AYAZ<sup>4</sup> Mehmet KARACA<sup>2</sup>

<sup>1</sup>Health Services Vocational College, University of Yuzuncu Yil, 65080Van / TURKEY

<sup>2</sup>Department of Internal Diseases, Faculty of Veterinary Medicine, University of Yuzuncu Yil, 65080 Van / TURKEY

<sup>3</sup>Refik Saydam Hygiene Center, ANKARA

<sup>4</sup>Department of Parasitology, Faculty of Veterinary Medicine, University of Yuzuncu Yil, 65080 Van / TURKEY

### SUMMARY

This study was carried out on sheep in order to determine the seroprevalence of toxoplasmosis in the province of Van. Sabin Feldman Dye Test (SFDT) was used to detect *Toxoplasma gondii* antibodies in 300 sheep obtained from the city center of Van and its regions: Erciş, Muradiye, Özalp, Edremit, Gürpınar and Gevaş. Out of the 300 sera examined, 162 (% 54.00) were negative and 138 (% 46.00) were positive at 1/16 and other dilutions. It was found that 47 sera sample (% 34.05) were positive at a 1/16 dilution, 64 sera sample (% 46.37) at a 1/64 dilution, 21 sera samples (% 15.21) at a 1/256 dilution and 6 sera sample (% 4.34) at a 1/1024 dilution. This results indicate that *Toxoplasma gondii* infection in sheep of Van province is widespread.

**Key Words:** *Toxoplasma gondii*, Sabin Feldman Dye Test, sheep

### Van Yöresi Koyunlarında Sabin-Feldman Dye Test ile *Toxoplasma gondii*'nin Seroprevalansı

#### ÖZET

Bu çalışma, Van yöresinde koyunlarda toxoplasmosisin seroprevalansını belirlemek amacıyla yapıldı. Van merkez ve ilçelerinden (Erciş, Muradiye, Özalp, Edremit, Gürpınar ve Gevaş) toplanan 300 koyun serumu Sabin Feldman Dye Test (SFDT) ile anti-*Toxoplasma gondii* antikolları yönünden incelendi. Serumların 162'si (% 54.00) negatif, 138'i (% 46.00) ise 1/16 ve üst titrelerde pozitif olduğu tespit edildi. Seropozitif titrelerin 47'si (%34.05) 1/16, 64'ü (%46.37) 1/64, 21'i (%15.21) 1/256 ve 6'sı (% 4.34) ise 1/1024 sulandırma basamaklarında pozitif bulundu. *Toxoplasma gondii* enfeksiyonunun Van yöresi koyunlarında yaygın olduğu görüldü.

**Anahtar kelimeler:** *Toxoplasma gondii*, Sabin Feldman Dye Test, koyun

### INTRODUCTION

Toxoplasmosis is a world-wide zoonosis of increasing concern in both human and veterinary medicine. The disease, caused by the obligate intracellular protozoon *Toxoplasma gondii*, is responsible for major economic losses in all classes of livestock through abortions, still birth and neonatal losses. It has a facultatively heteroxenous life cycle and can probably infect all warm-blooded animals and humans. Because of its great importance as a causative agent of a zoonosis, *T. gondii* has been studied most intensively among the coccidia (10, 16, 22)

The definitive hosts of the parasite are the domestic cat and other felines, the sexual cycle of it occurs only in these species (14). The disease is transmitted by the ingestion of oocysts (shed by infected cats) in contaminated food and water, or bradyzoites (cysts) in the tissues of an infected animal (10).

In humans, infection with *T. gondii* is normally chronic but largely asymptomatic, though it may cause stillbirth, blindness, mental retardation and occasional death of congenitally infected infants (13).

Toxoplasmosis is a generally mild, asymptomatic disease that begins with an acute phase, in which the tachyzoites of *Toxoplasma gondii* invade the cells, divide rapidly and trigger an efficient immune response in the host. A period of latency follows, during which the parasites form

intracellular cysts, containing slowly replicating forms of bradyzoites (10,16,22). Because of the lack of clinical symptoms in most of the cases, antenatal diagnosis mainly relies on serological investigations (10,16, 22, 25). Many tests are available for the detection of specific antibodies such as CF, IHA, IFAT, LAT, ELISA and MAT (the modified agglutination test) are used for diagnostic purpose(23). Among them the dye test (DT) described by Sabin and Feldman which is still considered as the 'gold standard'.

The frequency of infection is extremely variable in the different regions of the world. Several studies have been performed on *T. gondii* in the different parts of the world and prevalence of seropositivity was found 0-6.6 % in cattle, 22.6-33.2 % in sheep and 11.6-96 % in goats (4, 6, 12, 15, 17, 18, 24). In the studies on the seroprevalence of *T. gondii* in the different regions of Turkey had been reported as 12.1-63.1 % in goats, 7.1-88,7 % in sheep and 5.9-66 % in cattle (2, 8, 20, 21, 26).

In region of Van, very little study is known about the incidence of toxoplasmosis in man (5, 9). Although more livestock population in the province of Van is high, unfortunately, there is no study about seroprevalence of *T. gondii* on these animals. Therefore, its impact on the possible sources of infection to humans through domestic animals is not well established.

The aim of the present study was to obtain data on the prevalence of toxoplasmosis in different region of Van (the centrum of Van and it's provinces, Erciş, Muradiye, Özalp, Edremit, Gürpınar and Gevaş) by detection of antibodies developed against the parasite in the blood of sheep.

## MATERIALS AND METHODS

### Study areas

Blood samples were obtained from 300 sheep between January and May, 2001, taken from the animals living in the city center of the Van and its regions; Erciş, Muradiye, Özalp, Edremit, Gürpınar and Gevaş. Records of the animal locations, date of sample collection, the presence of cats and their accessibility to animal feed and water were recorded within each province.

### Animals

All animals were local breeds and ages ranging from 1 to 5 years, and were generally kept in small herds of 10 –50 animals during winter, however, pastured in the spring, summer and autumn. Approximately 10 ml of blood samples were taken from V. jugularis from each animals. Blood was transported to the laboratory of clinical science serum was obtained by centrifugation at 3000 x rpm for 20 minutes and stored at -20 °C until analysis.

### Serological assay

Sabin Feldman Dye Test (SFDT) has performed with vigorous antijen and metilen-blue dying according to the lab of Ankara Refik Saydam Hygiene Center (ARSHC) Contagious Diseases Research Department in Ankara (Sabin&Feldman, 1948). Three-four weekaged white Swiss-Albino mice were purchased from (ARSHC). As an activator serum, nonantibody of *T. gondii* and Mg, properdin, C2, C3, C4 rich human serum was used. As vigorous antigen, 48 hours passage of *T. gondii* Rh strain derived from mice periton fluid were used. He sera first inactivated in 56°C for 30 minutes, then diluted with %0.9 NaCl as 1/4, 1/16, 1/64, 1/256 and 1/1024 and stored in aliquots of 25 µl in eppendorfs tubes. 9.73 ml % 0.53 Na<sub>2</sub>CO<sub>3</sub>, 0.27 ml %1.91 Na<sub>2</sub>B<sub>4</sub>=7H<sub>2</sub>O prepared and 25 mg methylen blue added. With x40 objective, as antigen 25 vital *T. gondii* tachizoits in each microscopic area and in 25 µl activator serum were added to serum dilutions in the next tubes. The tubes were left to incubation for 50 minute. They have been kept in fridge for 10 minutes, after adding same amount alcohol methylen blue and assessed according to accepting the dye of *T. gondii* trophozoith with ligh microscope at x40 enlargement. It has been accepted toxoplasma negative if more than 50 % tachizoits didn't accept the dye in a microscopic area.

## RESULTS

Out of the 300 sera examined, 162 (% 54.00) were negative and 138 (% 46.00) were positive at 1/16 and other dilutions. It was found that 47 sera sample (% 34.05) were positive at a 1/16 dilution, 64 sera sample (% 46.37) at a 1/64 dilution, 21 sera samples (% 15.21) at a 1/256 dilution and 6

sera sample (%4.34) at a 1/1024 dilution. This results indicate that *Toxoplasma gondii* infection in sheep of Van province is widespread.

## DISCUSSION

*Toxoplasma gondii* is one of the most common parasitic infections world-wide with an estimated prevalence in 1–2 billion people (7). In ruminants infection not only results in significant reproductive and hence economic losses, but also has implications for public health since consumption of infected meat can facilitate zoonotic transmission. Human toxoplasmosis can be acquired both through ingestion of tissue cysts in undercooked meat and via ingestion of oocysts excreted from the definitive of *T. gondii*, the cat, although it is not known by which route infection is most commonly acquired (6).

The frequency of infection is extremely variable in the different regions of the world. Seroprevalence in the human population ranges from 0 to 90 % (11) and infection is more common in warm climates and in lowlying areas than in cold climates and mountainous regions, where conditions for sporulation and survival of oocysts are less favourable. The prevalence of *T. gondii* infections varies between ethnic group, and it is thought more effective sanitary and cooking habits rather than genetic differences (12). Because of consumption, a special food named "Çiğ Köfte" (a special raw ball prepared by hand-kneading and containing a mixture of lean meat, fine bulgur, and variety of species), undercooked meat habit is widespread in the region of Van. Another habit is that people in region of Van keep Van Cats in their houses.

In different countries, the prevalence of *T. gondii* in men has been reported from 10-93 % (1,3, 11) and 5-90 % in Turkey (5, 9). In spite of a high prevalence of *T. gondii* in Turkey, in the region of Van, it was found to be between 63.06-73.3 % in men (5, 9). But, there is no such study in animals in this region.

The prevalence of anti-*T. gondii* antibodies in ruminants has been reported in several countries. Studies in different countries have shown that the prevalence were between 22.6-33.2 % in sheep, 11.6-96 % in goats and 0-6.6 % in cattle (4, 17, 18). The seroprevalence of *T. gondii* in the different regions of Turkey had been reported as 7.1-88.7 % in sheep, 12.1-63.1 % in goats and 59-66 % in cattle (2, 8, 20, 21, 26). In the current study, it was found to be 34.6 % in sheep, 33.3 % in goats and 7.6 % in cattle. This result was similar to other findings from several countries of the world and Turkey.

This study was conducted to determine the prevalence of antibodies against *T. gondii* in sheep in the city of Van and its regions. With the current study, the prevalence of *T. gondii* was found to be 46.00 % in sheep. The parasite is a major cause of abortion and neonatal mortality in sheep and responsible for major economic losses in all classes of livestock through abortions, non ethiologic of abortion and still born can be caused of sheep in Van. It is believed that sheep remain chronically infected for life. Undercooked meat from infected sheep is an important source of infection for man. (12). The

high combined seroprevalence of anti-*T. gondii* antibodies for sheep may be due to the feed than the Van Cat which seen almost in every household. This indicated that exposure of sheep has potentially important implications for public health.

In conclusion, the results of this study confirm the presence of anti-*T. gondii* specific antibodies in sheep in the region of Van, Turkey. It will be necessary of toxoplasmosis on the animal conduct further studies to determine the impact of toxoplasmosis on the animal industry, and the potential risk of its transmission to humans through consumption of meat contaminated with tissue cysts of *T. gondii*.

## REFERENCES

1. Aspöck, H., Pollak, A. (1992): Prevention of prenatal toxoplasmosis by serological screening of pregnant women in Austria. Scand J. Infect. Dis. Suppl., 84: 32-38.
2. Babür, C., Esen, B., Brykoğlu, G. (2001): Yozgat'ta Toxoplasmosis gondii'nin seroprevalansı. Türk J. Vet. Anim. Sci., 25: 283-285.
3. Balows, A.H. (1991): Manual of Clinical Microbiology. 5 th. Ed., Washington.
4. Bekele, T., Kasali, O.B. (1989): Toxoplasmosis in sheep, goats and cattle in Central Ethiopia. Vet. Res. Com., 3: 371-375.
5. Berktaş, M., Baki, I., Yılmaz, H., Bozkurt, H. (1997): Çeşitli obstetrik sorunları bulunan kadın hastalarda Toxoplasma antikörlerinin araştırılması. T.Parasitol. Derg., 21:360-362.
6. Bisson, A., Maley, S., Rubalre-Akiiki, C.M., Wastling, J.M. (2000): The seroprevalence of antibodies to Toxoplasma gondii in domestic goats in Uganda. Acta tropica., 76: 33-38.
7. Chang, H.R. (1996): The potential role of azithromycine in the treatment or prophylaxis of toxoplasmosis. Inter. J. STD and AIDS., 7:18-21.
8. Çiçek, H., Babür, C., Kener, B. (2000): Afyon mezbahasında kesilen mandalarda (Anatolian buffalo) Anti-Toxoplasma gondii antikörlerinin Sabin-Feldman Dye Testi ile (SBDT) araştırılması. II. Ulusal Tropical Hastalıklar Kongresi, 25-29 Eylül, Şanlıurfa.
9. Değer, S., Cantoray, R., Gül, A., Akdemir, C. (1995): Toxoplasmosis de IgG ve IgM seviyelerinin ELISA ile ölçümü ve bu testin tanı için önemi. Yüzüncü Yıl Üniv. Vet. Fak. Derg., 6:34-37.
10. Dubey, J.P. (1998): Advances in the life cycle of toxoplasma gondii. International J.Parasitol., 28: 1019-1024.
11. Dubey, J.P., Beattie, C.P. (1988): Toxoplasmosis of animals and man, 1 st ed. CRC Press, Boca Raton,FL.
12. Esteban-Redondo, I., Maley, S.W., Thomson, K., Nicoll, S., Wright, S., Buxton, D., Innes, E.A. (1999). Detection of *T. gondii* in tissues of sheep and cattle following oral infection. Vet. Parasitol., 86: 155-171.
13. Frenkel, J.K. (1988): Physiopathology of toxoplasmosis. Parasitol. Today., 4: 273-278.
14. Frenkel, J.K., Dubey, J.P., Miller, N.L. (1970): Toxoplasma gondii in cats: Fecal stages identified as coccidian oocysts. Science., 167: 893-896.
15. Hashemi-Fesharki, R. (1996): Seroprevalence of toxoplasma gondii in cattle, sheep and goats in Iran. Vet. Parasitol., 61:1-3.
16. Levine, N.D. (1987): Veterinary Protozoology. Iowa State University Press, Ames., USA.
17. Pleva, J., Skol, J., Cabataj, R., Saladiova, D. (1977): Epizootic and epidemiological significance of toxoplasmosis. Slovensky-Veterinarsky-Casopis., 22:127-129.
18. Polydorou, K. (1989): Cyprus Annual report of the Department of Veterinary services for the year 1988. Source (Bibliographic citation ), (VETCD, 1998-1999/02).
19. Sabin, A.B., Feldman, H.A. (1948). Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (Toxoplasma). Science., 108: 660-663.
20. Sevinç, F., Dik, B., Gökçen, A., Altınöz, F., Uslu, U. (2000): Konya yöresi keçilerinde Toxoplasma gondii'nin İHA (İndirekt Hemaglutinasyon) ve İFA ( İndirekt Floresan Antikor) Testleri ile seroprevalansı. T. Parasitol. Derg., 24: 57-60.
21. Sevinç, F., Birdane, F.M., Evinç, M., Dik, B., Altınöz, F. (2000): Konya yöresi sığırlarında Toxoplasma gondii'nin İHA (İndirekt Hemaglutinasyon) ve İFA ( İndirekt Floresan Antikor) Testleri ile seroprevalansı. T.Parasitol.Derg., 24: 176-179.
22. Soulsby, E.J.L. (1986): Helminths, Arthropods and Protozoa of Domesticated Animals. Seventh ed., Bailliere Tindall, London.
23. Tanyüksel, M. (1994): Toksoplazmosis tanısında serolojik testlerin karşılaştırılması. T.Parasitol.Derg., 18: 266-274.
24. Van Der Puije, W.N.A., Bosompem, K.M., Canacoo, E.A., Wastling, J.M., Akanmori, B.D. (2000): The prevalence of anti-Toxoplasma gondii antibodies in Ghanaian sheep and goats. Acta Tropica., 76: 21-26.
25. Wastling, J.M., Nicoll, S., Buxton, D. (1993). Comparison of two gene amplification methods for the detection of Toxoplasma gondii in experimentally infected sheep. J. Medical. Microbiol., 36: 360-365.
26. Yıldız, K., Babür, C., Kılıç, S., Aydenizöz, M., Dalkılıç, İ. (2000): Kırıkkale mezbahasında kesilen koyun ve sığırlar ile mezbaha çalışanlarında anti-toksoplazma antikörlerinin araştırılması. T.Parasitol. Derg., 24: 80-185.