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ORİJİNAL MAKALE

# Investigation on the Seroprevalence of *Toxoplasmosis*, *Listeriosis* and *Brucellosis* in Goats living in the region of Van, Turkey

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***Summary:*** *This study was carried out on goat sera to determine the seroprevalence of listeriosis, toxoplasmosis and brucellosis in the province of Van. Sabin Feldman Dye Test (SFDT), Osebold Agglutination Test (OAT) and Micro Agglutination Test (MAT) were applied to determine antibodies developed against Toxoplasma gondii, Listeria monocytogenes and Brucella melitensis respectively. Out of the 98 sera, 80.61% toxoplasmosis, 34.69% listeriosis and 6.12% brucellosis were evaluated as positive. This results indicate that Toxoplasmosis is much more widespread than Listeriosis, and Brucellosis in goats living in the province of Van.*

***Key words: Toxoplasma, Listeria, Brucella, serology, seroprevalence, goats***

***Van Yöresi Keçilerinde Toxoplasmozis, Listeriozis ve Brucellozis'in Seroprevalansı Üzerine Araştırmalar***

***Özet:*** *Bu çalışma Van yöresi keçilerinde toxoplasmosis, listeriosis ve brucellosis seroprevalansını belirlemek için yapıldı. Toxoplasma gondii, Listeria monocytogenes ve Brucella melitensis'e karşı oluşan antikorları belirlemek için sırası ile Sabin Feldman Dye Testi (SFDT), Osebold Agglutination Testi (OAT) ve Micro Agglutination Testi (MAT) uygulandı. 98 serum örneğinin, %80.61 toxoplasmosis, %34.69 listeriosis and %6.12 brucellosis yönünden seropozitif olduğu tesbit edildi. Bu sonuçlar ile Van yöresi keçilerinde Toxoplasmosisin, listeria ve brucella'ya göre çok daha yaygın olduğu görüldü.*

***Anahtar kelimeler: Toxoplasma, Listeria, Brucella, seroloji, seroprevalans, keçi***

## INTRODUCTION

In goat breeding, most important problem is infectious diseases. Main infectious diseases are toxoplasmosis, listeriosis and brucellosis which all cause abortion apart from other symptoms (1, 5, 9, 12, 17, 20, 28).

Toxoplasmosis is a zoonosis with an infectious reservoir encompassing all animals. As the definitive host, the domestic cat appears to be the major culprit in transmission to other animals. After infecting a new host, including acute, latent, and reactivated disease as well as congenital disease and chorioretinitis (20, 21). Acute acquired toxoplasmosis is most commonly asymptomatic, but it can range from mild symptomatology in the normal host to fulminant and fatal illness in the immunocompromised host. After developing an immunologic response to the initial acute infection, most normal hosts contain the infection. The prevalence of seropositivity for Toxoplasma antibodies varies with geographic location, flock and country (9, 16, 20, 36).

*Listeria monocytogenes* is a gram positive, facultative, intracellular bacteria which is ubiquitous in nature and cause septicemia, mastitis, encephalitis and abortions in ruminants. It has emerged as one of the most important food-borne pathogens and occurs in variety of food items such as milk and dairy foods (23), seafood, raw as well as cooked egg (15) and foods of animal origin (7,27). The main route of transmission of *L. monocytogenes* is by ingestion of contaminated food and the disease is particularly common in ruminants fed on silage (39, 23). The authentic diagnosis of listeriosis has been made by isolation of the organism but it is time-consuming (24). The detection of antibodies as an indirect indication of infection among animals and man have the advantage of screening large population in a

relatively short time. The conventional tests employ crude listerial proteins such as heat-killed, trypsinized or cold-extracted antigens to detect listerial antibodies through a battery of serological tests such as the complement fixation test, agglutination, immunoprecipitation and passive immunohaemolysis (2,22,25,29).

Brucellosis is also major zoonotic problem in many countries, and its eradication in the animals is a necessary step to control the human disease. Brucellosis in goats, caused by *Brucella melitensis*, frequently results in abortions and diminished levels of milk production (10, 17, 32). Brucellosis can be considered as a great challenge to the development of dairy production in developing countries. It is one of the most economically devastating diseases, which causes great losses among the offspring and causes health problems in the rural and urban population, due to either contact with the infected materials or consumption of the contaminated dairy products (1, 10, 19, 17). Complement Fixation Test (CFT), Rose Bengal Test (RBT), and the Serum Agglutination Test (SAT) are among the most useful tests for routine diagnosis (10,17,28,30). Furthermore, currently brucella Micro Agglutination Test (MAT) has been used to detect brucellosis serologically in this country which found to be quite specific and sensitive in the serological diagnosis of brucellosis (6,10,14,17).

Goat production in this region is an important livelihood source. The region is a border city which from time to time animal crossing from other countries such as Iran, Iraq and can not be controlled properly. Thus, epidemic in this region occur more often. Problems effecting goats health is also affecting their owners economically. Therefore, in this study the seroprevelance of the most important diseases causing abortion in goats were aimed to investigate.

## MATERIALS AND METHODS

### *Blood Samples*

A total of 98 female goats at different age were examined clinically and blood samples were taken. Serum samples were obtained from these blood samples and stored at −20°C until tested. These goats were chosen because abortion in this flocks were observed in the earlier pregnancy period. All the tests for toxoplasmosis, listeriosis and brucellosis in the serum samples taken in the present study were carried out by the Laboratories of Refik Saydam National Hygiene Center, Department of Communicable Diseases Research, Ankara.

### *Serological assays*

All of the sera were tested for antibodies against *Toxoplasma gondii, Listeria monocytogenes* and *Brucella melitensis*.

### *Sabin-Feldman Dye Test (SFDT) for Toxoplasmosis*

Serum samples were tested for anti-T.gondii antibodies in fourfold dilutions (1/16; 1/64; 1/256; 1/1024) using the standard Sabin-Feldman dye test (SFDT) as routinely performed according to the modified method of Feldman and Lamb (16). The SFDT result was regarded as positive if more than 50% of tachyzoits did not accept the dye (unstained) at ≥1:16 examined under the light microscope (x 400).

### *Antigen preparation and agglutination test for Listeriosis*

An antibody titration test to detect antibodies for L. monocytogenes was carried out according to the method described by Osebold et al. (29). The test antigen used in the present study was prepared in the Laboratories of Refik Saydam National Hygiene Center, Department of Communicable Diseases Research, and the assay was carried out in 3 steps. For the first step, the whole cell antigens were prepared from Staphylococcus aureus (ATCC 29213) strains by the Osebold method. In the second step, Listerial antigens were prepared from L. monocytogenes 1/2a, 1/2b, 4b, 4c and 4d strains and were combined in the same suspension. In the last step an agglutination test was performed after the absorption of sera samples with S. aureus antigen.

### *Brucella Micro Agglutination Test (MAT)*

The MAT was performed as described by Baum. et al. (6). Briefly, Two-fold serial dilutions of sera, ranging from 1:2.5 to 1:40, were prepared in saline and 0.5% phenol in V-shaped microtiter plates. Fifty µl of B. abortus S99 antigen solution stained with Safranin-O (0.02%) composed of 5%NaCl and 0.5% phenol was added to each well containing 50 µl diluted serum and the plate was covered with a lid. The negative control wells contained phenol saline and the antigen. The results were read after 18 h of incubation at 37°C. The agglutination results were considered as negative (compact red dot) or positive (large diffuse red mat). Positive and negative controls were run for each test.

***Statistical Analysis***

Descriptive statistic and $X^2$ test were used in the serum samples to determine positivity and mix antibody titers (11).

**RESULTS**

***Clinical findings***

Clinically examined goats had no specific clinical signs of the diseases. But in their history; the flocks suffered from abortus in their previous pregnancy period.

***Serological findings***

According to Sabin-Feldman Dye Test (SFDT); out of for 98 goat 79 (80.61%) were seropositive for toxoplasmosis at ≥1:16 dilutions. Seropositivity distribution at different dilutions were 42 (42.85%) at 1/16 dilution, 27 (27.55%) at 1/64 dilution, 7 (7.17%) at 1/256 dilution and 3 (3.06%) at 1/1024 dilutions. Furthermore, 34 samples (34.69%) were seropositive for listeriosis according to Osebold Agglutination Test (OAT). MAT for brucellosis also revealed 6 (6.12%) seropositivity at ≥1/20 dilutions. Additionally, 32(32.6%) seropositivity to both toxoplasmosis and listeriosis, 7(7.14%) seropositivity to both toxoplasmosis and brucellosis, 3 (3.06%) seropositivity to both listeriosis and brucellosis, 3 (3.06%) seropositivity to all toxoplasmosis, listeriosis and brucellosis were determined.

**DISCUSSION**

Infectious diseases causing abortus such as toxoplasmosis, listeriosis and brucellosis are important diseases for goats (1,10,20). They cause important economical losses, not only by abortus but also reduction in milk production. Especially the goat production is one of the most important income for the farmers. Their importance does not come only from their effect on animal, they also contaminate human beings (21,33,34).

Although seropositvity to an organism does not translate into verification that the animal was clinically affected by that organism, the infectious diseases; toxoplasmosis, listeriosis and brucellosis seem to be widespread among goat flock from the region of Van and probably represents an important factor that contributes to the decreased productivity of those animals (35,36). Most probably decreased goat production in Turkey has been occurring year by year due to such diseases. Unfortunately, there aren't many studies concerning these diseases surveillance. Therefore, these disease prevalence need to be put forward to combat and to make proper eradication programs.

Studies concerning toxoplasmosis have been made on several animal species including on goats world wide (21,26,36). Several serological tests have been used to detect antibodies against toxoplasmosis. Many tests are available for the detection of specific antibodies to toxoplasmosis (16, 20, 26). One of them is SFDT, which is used for diagnostic purpose and considered a gold standard test. In the present study, seropositivity to toxoplasmosis was found to be 80.61% using this test. The frequency of toxoplasmosis infection is extremely variable in the different regions of the world. Several studies have been performed on toxoplasmosis in different parts of the world and prevalence of seropositivity was found 11.6-96% in goats (9, 20, 26). In the studies on the seroprevalence of toxoplasmosis in the different regions of Turkey had been reported between 12.1-63.1% in goats ( 35, 36). It can be seen from our results that findings reported in this study with regard to toxoplasmosis was quite high in comparison to results given for average Turkey's results. This could be due to the animals owners unawareness to the vaccination programme and also the regions characteristics. The region is border to Iran and Iraq which illegal animal trade occur from time to time. Therefore, high seropositivity could also be relied on this stuation.

In the present study seropositivity to listeriosis was found to be 34.69% in goats. Epidemiological studies have revealed that only Listeria monocytogenes and only strains belonging to serotypes 1/2a, 1/2b and 4b were implicated in 90% of outbreaks of listeriosis (18). Therefore in the present study antibodies against listeria monocytogenes were investigated using OAT test. In recent years PCR and ELISA tests have widely been carried out in the diagnosis of listeriosis (3,5,12,13). But, OAT test has also been used safely especially in epidemiological screenings (29). Listeriosis is mainly transmitted by ingestion of contaminated food and the disease is particularly common in ruminants fed on silage (8,31). However, goats used in the present study have never been had silage according to anamnesis. According to different serological test; the seroprevalence of listeriosis in goats have been reported to be between 14.52% and 41.13% (4, 5, 31). Findings reported in

the present study were also in these limits. In a study carried out by Tütüncü et al. (37) found the seroprevalance of listeriosis in cattle as 28.5% in the region of Van. In addition, Erdoğan et al.(13), studied seroprevalance of listeriosis in cattle and found 88.7% using ELISA in the region of Kars. But, we couldn't sight any seroprevalance studies on goat in Turkey. Therefore, findings in this study should be considered as novel results. Furthermore, studies concerning listeriosis in this region (which is a border region) have not been investigates before.

Brucella melitensis is the most common agent of caprine and human brucellosis. Therefore, small ruminants are considered the main source of human infection (26,32,34). Brucellosis is worldwide disease particularly in Near East countries, middle East, Iran, Iraq, Turkey including province of Van (32, 34). It is one of the most economically devastating disease, which causes great losses among the offspring and causes health problems in rural and urban population, due to either direct contact with infected materials or consumption of the contaminated dairy products such as milk (32). Eradication of this disease in the animals is a necessary step to control the human disease. In the European Union, this disease is limited to Mediterranean countries. Although the disease seroprevalence in sheep have been reported to be quite high in Turkey, Its serological presence in goat has been reported to be low in comparison to sheep (28,32,34). In the present study the seroprevalence of brucellosis in goats were found to be 6.12%. The test used in the present study to determine seroprevalance of brucellosis was MAT which has widely been used in Turkey for screening brucellosis (14). On the other hand, screening brucellosis in Turkey has been concentrated mainly on sheep and cattle (19). Furthermore, brucella screening on goats in our region (which is more vulnerable) have not been carried out according to our knowledge so far. Therefore, this study concerning brucellosis in this region should be considered as novel findings.

As a result, with these findings, seroprevalance of the diseases causing aborts in goats were found to be quite high in this region. Furthermore, results showed that the seroprevalance of toxoplasmosis was the highest. These diseases are very important with regard to economical losses. Therefore, to eradicate such disease and to reduce economical losses, more detailed studies should be made in this region to make proper and effective challenge.

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