A study on presence of brucellosis in milk from Afyon region sheep

**B. Kenar**†1, S. Erdenlig2, E. Şengör2

1-Asist. Prof. Dr., University of Afyon Kocatepe, Faculty of Veterinary Medicine, Dept. of Microbiology. Afyonkarahisar- Turkey; 2- Dr. Pendik Veteriner Kontrol ve Araştırma Enstitüsü-İstanbul- Turkey; 3-Assoc. Prof. Dr., University of Afyon Kocatepe, Faculty of Veterinary Medicine. Dept. of Animal Husbandry, Afyonkarahisar - Turkey

**SUMMARY**

The aim of this experiment is to determine the brucellosis incidence from transmission to human by the consumption of cheese made with raw milk from Afyon region sheep which was contaminated with sub-clinically infected animals’ milk.

Milk from one lactation period of 1100 sheep was tested with California Mastitis test (CMT).

Milk samples from 100 sub-clinically infected sheep were collected in sterile screw-capped tubes and tested with Milk Ring Test (MRT). Sixteen milk samples were found to be positive regarding *Brucella*.

The positive animals were also tested microbiologically by monospecific antiserum agglutination test and *Brucella melitensis* type 3 was isolated and identified from 4 of them.

These milk samples were also tested regarding somatic cell count (SCC) and 800.000 to 10.000.000 cells per ml were determined. There appeared to be some sort of parallelism between MRT, isolation and SCC results.

Key words: Sheep, brucellosis, milk ring test, isolation, somatic cell count., mono specific antiserum

**INTRODUCTION**

Sheep never been milked in most of the countries. Milk generally only aimed for feeding the baby lambs. Contrary to this, sheep is milked in many region of Turkey since its high protein, fat and dry matter contents and also distinctive aroma and flavor compared to cow milk in order to make yogurt, cheese, butter and other dairy products (6, 7).

† Corresponding author e-mail: bkenar@aku.edu.tr, beytullahkenar@yahoo.co.uk
Brucellosis, Campylobacteriosis, Salmonellosis, Chlamydiosis, Tuberculosis and Listeriosis are among the main abort causing diseases (5, 7, 12). Since these are the zoonotic diseases, they can also infect the human. Brucellosis is the most prominent disease in the world and Turkey (5, 6, 9, 10, ).

When the sheep abort they can shed Brucella bacteria (200,000/ml of milk) by their excreta and secretes (5, 12, 13, 16). It means that those sheep can shed brucella bacteria with their milk as well.

Brucella agents can contaminate the healthy animals in sheep-folds and pastures, and human during milking or consumption of raw milk and dairy products made by raw milk. The role of carrier and reservoir animals are high in disease spread. The Brucella can be transmitted to human by the diseased animal’s milk or dairy products made by this milk or healthy milk mixed with such milk without pasteurization.

The Ministry of Agriculture and Rural Affairs (3) has launched a program screening brucellosis countrywide since 1984. Under this screening framework early age vaccination is made only to lambs and kids while adult vaccination only carried out to the healthy animals at the places where the disease outbreaks (4).

MATERIAL AND METHODS

The material of this study is 100 milk samples obtained from 1100 sheep inspected for brucellosis with CMT. The udders of each sheep are washed with lukewarm water and dried with clean paper tissues and disinfected by suitable disinfectants before milking. The udders then wiped with ethyl alcohol. The first milk in the teat channel is milked into another container and discarded. Two ml of milk from both udders of each sheep then milked into CMT container and equal amount of CMT reagent is added (20 g. Sodium Laurile Sulfate + 15 cc of 10% NaOH solution + 0.1 g. Brome Cresol Purple + 1000 cc. distilled water) and shaken with rotational movement.

The teats of the sheep with positive CMT is wiped with 70% ethyl alcohol soaked cotton wool and dried with clean paper tissue towel. Eight to ten cc of milk samples are collected into sterile screw capped tubes, shaken well with vortex to homogenize and 0.01 ml of the content is spread over 1 cm² surface area of a microscope slide by sterile loop, dried and stained (6) in order to count the somatic cells (8, 10, 13).

Another sample of 1 ml homogenized milk is taken from the same screw capped tubes into agglutination tubes (75x10 ml) and 1 drop of MRT antigen (obtained from Pendik Veterinary Control and Research Institute) added, shaken well and incubated at the 37°C incubator for 1.5 hours and the results were interpreted (2, 4, 5). MRT positive milk samples are cultured in enriched blood agar and Brucella agar (Oxoid) as a set and one set is placed in anaerobic media jar (10% CO₂-Gas Generating Kit, Oxoid Br-39) while the other set is incubated in aerobic environment for 3-5 days. The isolated colonies were identified.
morphologically by microscopic and macroscopic way and also by classic way (2, 5, 13). Monospecific antiserum agglutination test (obtained from Pendik Veterinary Control and Research Institute) is made in order to identify the Brucella (8, 13).

**RESULTS**

Milk samples of 100 sheep were found to be positive regarding CMT among the tested 1100 sheep. Sixteen samples out of 100 were positive with MRT and four *Brucella melitensis* type-3 were isolated and identified by monospecific antiserum agglutination test. As somatic cell count, 800,000-10,000,000 cells were found.

**DISCUSSIONS AND CONCLUSIONS**

Sheep brucellosis remains as one of the most important zoonotic disease in the many countries around the world including Turkey. Since it is a zoonotic disease, it can be transmitted to human via the consumption of milk and dairy products made especially from unpasteurized milk.

It was reported that the somatic cell counts from sheep and goat milk was similar to those obtained from cows’ milk and was between 600,000 and 5,700,000 in 1 ml of milk (6, 10).

Baysal and Kenar (7) reported sub-clinic mastitis in 256 among 3627 sheep investigated and they isolate and identify 2 *Br. Melitensis*.

Grain-Bastuji et al (11) reported that 4 *Brucella melitensis* were isolated and identified out of 16 MRT positive samples in sheep, however they also reported that MRT tests in sheep was not as sensitive as that of cow supporting the report made by Okoh (14). Okoh found 5 *Br. abortus* among 22 MRT positive sheep in Nigeria.

Abd-el Ghani et. al (1), reported that out of 339 milk samples, 2.31% were positive with SAT, 2.44% positive with CFT and 2.06% positive with MRT regarding brucellosis in Egypt and the results were in harmony.

It appeared that our results in this experiment were supported by the above mentioned reports.

Brucella agents may remain infectious from 90 days to 200 days in cheese depending on the type of the cheese produced (15, 16, 17). In order to reduce the prevalence of brucellosis and to increase the public awareness, regional control programs should be implementing and mass media communication means should be put in use to reduce the usage of raw milk for the production of the dairy products.
REFERENCES