

Investigation of *Brucella abortus* and *Brucella melitensis* at Cheeses in Afyonkarahisar, Turkey

¹Recep Kara and ²Levent Akkaya

¹Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03000 Afyonkarahisar, Turkey

²Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Balıkesir University, TR-10440 Balıkesir, Turkey

Abstract: *Brucella* contaminates people with raw milk, raw dairy products and especially with cheese made from raw milk. This study has aimed to detect *Brucella abortus* and *Brucella melitensis* in fresh cheese (Coban Cheese, 100-mold), traditional Afyon Tulum Cheese (Aged Skin Bag Cheese) (50-mold) and sheep-lamb tulum (50-mold) samples provided from Afyonkarahisar province. Conventional Culture Method has been used in the isolation and identification of *B. abortus* and *B. melitensis*. In the case study, *Brucella* spp. has been detected at the rates of 9% (2% *B. abortus*, 7% *B. melitensis*) in the analyzed fresh cheese samples and 6% (2% *B. abortus*, 4% *B. Melitensis*) in Afyon Tulum Cheese. However, *Brucella* spp. has not been detected in analyzed sheep-lamb tulum samples. Consequently, it has been accepted that fresh cheese and Afyon Tulum Cheese samples are contaminated with *B. abortus* and *B. melitensis* which impose a high risk for the public health.

Keywords: Afyon tulum cheese, *Brucella abortus*, *Brucella melitensis*, *Brucella* spp., cheese

INTRODUCTION

Brucellosis in humans and animals is a zoonotic infection caused by *Brucella* spp. in many countries. Infection in humans is known as “Malta Fever” or “Wave of Fever” (Arda *et al.*, 1997). It has been reported that although the infection is seen in the sheep, goat, cattle, buffalo, dog, other farm animals and wild animals; wild animals can act as a reservoir for domestic animals. Brucellosis is spread throughout the world and is endemic in some developed countries (Trujillo *et al.*, 1994). Brucellosis is accepted as a frequent zoonotic infection by WHO (World Health Organization), FAO (Food and Agriculture Organization) and OIE (World Organization for Animal Health) (Yüce and Çavuş, 2006). In our country, notification of Brucellosis, both in humans and animals, is obligatory. According to Notification of Communicable Diseases and the Notification System Standard Diagnostic and Laboratory Guide published by T.R. Ministry of Health General Directorate of Primary Health Care, notifiable diseases are divided into four groups and Brucellosis is among “Group a Notifiable Diseases” (Anonymous, 2011). In Turkey, Brucellosis is the most common infectious disease in rural areas (Bodur *et al.*, 2003), especially in Southeast Anatolia, Eastern Anatolia and Central Anatolia regions in order of frequency (Kaya, 2006).

Disease can transmit to humans through the consumption of raw or unpasteurized milk and dairy product, direct contact with infected carcass, infected uterus and discharges or inhalation of contaminated dust (Nielsen *et al.*, 1996). *Brucella* is seen in veterinarians, livestock producers, animal product processing and packaging process workers and laboratory workers (Ruben *et al.*, 1991).

In Mediterranean countries, a significant proportion of cheeses are made from raw milk (Trujillo *et al.*, 2002). The desired flavor and aroma of cheese are much more favorable in raw milk cheeses than in the ones made from pasteurized milk (Gaya *et al.*, 1990). Nowadays, producing cheese from raw milk has rallied again due to immense demand of flavor and aroma from consumers. However, there is an increasing food security problem in relation to the cheese made from raw milk (De Buyser *et al.*, 2001). Cheeses made from raw milk are in the category of the dangerous food in recent years (West, 2008). It has been reported that consumption of the cheese made from the milk of infected animals acts an important role in the transmission and spread of Brucellosis to humans (Ataş *et al.*, 2007). So, the purpose of this study was to investigate the presence of *Brucella abortus* and *Brucella melitensis* in fresh cheese (coban cheese), traditional Afyon Tulum Cheese (Aged Skin Bag Cheese) and sheep-lamb tulum samples provided from Afyonkarahisar market.

Corresponding Author: Recep Kara, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03000 Afyonkarahisar, Turkey

MATERIALS AND METHODS

To examine the presence of *Brucella abortus* and *Brucella melitensis* in fresh cheese (Çoban cheese), traditional Afyon Tulum Cheese (Aged Skin Bag Cheese) and sheep-lamb tulum samples provided from Afyonkarahisar market; 100 molds of fresh cheese (Çoban cheese), 50 molds of Afyon Tulum Cheese and 50 molds of sheep-lamb tulum samples have been used as the material.

Extracting samples: Cheese samples about 100 g were put into sterile plastic sample containers were brought to the lab under cold chain and were analyzed.

Identification of *Brucella abortus* and *Brucella melitensis* presence in the samples: Farrell (1974) was used for the isolation and identification of *Brucella abortus* and *Brucella melitensis*. For this purpose, a cheese sample of 10 g was homogenized in 90 mL of Farrell's broth (*Brucella* Broth BBL 4311088, 5% at serum Oxoid SR 35, 10 g/L Glikoz Merck 1.08346.1000, 1 vial/500 mL *Brucella* Selective Supplement Oxoid SR83); one of the samples was incubated under aerobic conditions while the other was incubated in the medium of 10% CO₂ at 37°C for 5 days.

“Wet-Dry Swap Procurement Method” was used to extract samples from sheep-lamb tulum (Anonymous, 1987). By this method, the surface of the samples was determined as 40 cm² with sterile gauge and 2 buffers were used for each sample surface. After the first swap was immersed into sterile NaCl (Merck) solution of 0.85%, the second one was dry-scuffed on the same determination surface for 20 sec. Later, the swaps were combined; the ends were separated from the combination and were put into previously prepared tubes of *Brucella* Broth (BBL 4311088; *Brucella* Selective Supp. Oxoid SR 83; 5% at serum Oxoid SR 35, 10 g/L Glikoz Merck 1.08346.1000) of 10 mL. One of the tubes was incubated in aerobic atmosphere and the other was incubated at the medium of 10% CO₂ at 37°C for 5 days.

After incubation, 0.1 mL of each homogenized was inoculated to Farrell's Agar (*Brucella* Medium Base Oxoid CM169, 5% at serum Oxoid SR 35, 1 vial/500 mL *Brucella* Selective Supplement Oxoid SR83) surface and one of the Petri was incubated under aerobic conditions while the other was incubated at the medium of 10% CO₂ at 37°C for 5 days. After incubation, translucent and pale yellow colonies in diameter of 1-2 mm with fully rounded edges were selected; agglutination tests like gram staining, H₂S formation, urease activity, catalase activity, *B. abortus* Monospecific A antiserum (RHSM 140.215) and *B. melitensis* Monospecific M antiserum (RHSM 140.217) were performed on all colonies.

Table 1: *Brucella abortus* and *Brucella melitensis* in fresh cheese, Afyon tulum cheese and sheep-lamb tulum samples

Numune (n*)	<i>Brucella abortus</i> (%)	<i>Brucella melitensis</i> (%)	Total (%)
Fresh cheese (çoban cheese) (100)	2 (2%)	7 (7%)	9 (9%)
Afyon tulum cheese (50)	1 (2%)	2 (4%)	3 (6%)
Sheep-lamb tulum (50)	0 (0%)	0 (0%)	0 (0%)
Total (200)	3 (1.5%)	9 (4.5%)	12 (6%)

n*: Number of samples

RESULTS

In this study, 100 molds of fresh cheese (Çoban cheese), 50 molds of Afyon Tulum Cheese and 50 molds of sheep-lamb tulum samples from Afyonkarahisar market were analyzed to detect the presence of *Brucella abortus* and *Brucella melitensis*. The results of the analysis were shown in Table 1. *Brucella* spp. was detected in 9 (9%) out of 100 molds of fresh cheese samples and 2 (2%) of the isolates were identified as *B. abortus* and 7 (7%) were identified as *B. melitensis*. However, *Brucella* spp. has not been detected in analyzed sheep-lamb tulum samples.

DISCUSSION

The main cause of the spread of *Brucella* spp. infection among people, especially in rural areas, is infected food like raw milk and cheese, cream and butter made from raw milk (Sözen, 1996; Erol, 1997). *Brucella* infections are seen 4 times more during the summer as a result of the increasing opportunity of finding fresh milk and milk products like cheese and cream during people's travel to rural areas (Sözen, 1996). Many residential units around Afyonkarahisar earn their living on milk and dairy products; hence, Brucellosis is a risk for the residents (Altundiş, 2001).

In this study, *Brucella* spp. was reported for fresh cheese samples at the rate of 9% (2% *B. abortus*, 7% *B. melitensis*) and 6% (2% *B. abortus*, 4% *B. melitensis*) for Afyon Tulum Cheese samples. *Brucella* spp. has not been detected in analyzed sheep-lamb tulum samples. Among the national studies on *Brucella* spp. presence in the cheese, Tunçbilek (1992) isolated approximately 4% *Brucella* spp. out of 100 white cheese samples and identified 1% as *B. abortus* and 3% as *B. melitensis* in Ankara. In the study they conducted in Van, Sancak *et al.* (1993), isolated *Brucella* spp. at the rate of 17.5% out of 40 fresh herby cheese and they identified 15% of the strains as *B. melitensis* and 2.5% as *B. abortus*. In their Elaziğ study, Patır and Dinçoğlu (2001) reported *Brucella* spp. at the rate of 3.3% out of 30 white cheese samples and 1.8% out of 55 tulum cheese samples. Kalender *et al.* (2001) isolated

Brucella spp. from 20.5% of 78 fresh tulum samples they gathered from Elazığ, Erzincan and Tunceli and 81.3% of the isolates were *Brucella melitensis* while 18.7% were *B. abortus*. Güllüce *et al.* (2003) collected 120 white cheese, 60 Çivil Cheese and 52 quark cheese samples from Erzurum and they detected *B. abortus* antigen at the 21, 66% of the white cheese samples. They did not report *B. abortus* antigen in Çivil or quark cheese samples. Alim and Tomul (2005) analyzed fresh cheese samples in Sivas between 2003 and 2004 and they detected *Brucella* spp. at the rates of 7.1 and 8.5%, relatively. Ataş *et al.* (2007) isolated *Brucella* spp. at the 5.9% of 135 fresh white cheese samples in Sivas. (2.9%) of the isolates were *B. melitensis* and 2.9% were *B. abortus*.

The rate of *Brucella* spp. detected in this study was lower than the rates determined by Sancak *et al.* (1993), Kalender *et al.* (2001) and Güllüce *et al.* (2003); however the rate was higher than the ones determined by Tunçbilek (1992), Patır and Dinçoğlu (2001), Alim and Tomul (2005) and Ataş *et al.* (2007).

The differences between the rates of *Brucella* spp. might stem from the source of the milk used, the level of the contamination, changes in production processes, production circumstances, use of raw milk, time in brine and duration of maturation of the cheese. Non-detection of *B. abortus* and *B. melitensis* in sheep-lamb tulum samples may result from cleaning and washing phases of the skins.

The hygienic quality of the raw milk produced in our country is not adequate for cheese production. However, due to the high demand from the consumers who prefer traditional and tasty cheese, the production of cheese from raw milk is still widespread. In this study, fresh cheese (Çoban cheese) and Afyon Tulum Cheese samples provided from Afyonkarahisar market were reported to be contaminated with *B. abortus* and *B. melitensis*. The main factor for this detection is probably the use of raw milk contaminated with *Brucella*. Another significant factor could be cross-contamination as a result of unhygienic production circumstances. Also, one of the reasons why *B. abortus* and *B. melitensis* were detected in Afyon Tulum Cheese might be the marketing of the cheese before maturation.

CONCLUSION

As a conclusion, in order to prevent the risk of *Brucella* infection resulting from the consumption of fresh cheese and Afyon Tulum Cheese and to achieve the concept of "From Dairy to the Table", HACCP (Hazard Analysis and Critical Control Points), GMP (Good Manufactured Practice) and GHP (Good Hygiene Practice) should strictly be applied; herd health should regularly be controlled against; direct contact with contaminated materials should be avoided

and contaminated materials should be eliminated immediately; necessary educations to raise awareness of the public and the producers should be provided; raw milk with a High microbiological quality or pasteurized milk should be used in cheese production; the cheese should be produced under modern and hygienic conditions and facilities should be investigated frequently; the uncontrolled and uninvestigated sale of cheese made in the open should be prevented; cheese should be consumed after necessary maturation period.

ACKNOWLEDGMENT

We would like to thank the Afyon Kocatepe University Research Foundation (Project Code: 09-VF-19) for financially supporting this study.

REFERENCES

- Alim, A. and Z.D. Tomul, 2005. Investigation of brucella in the fresh cheese samples sold at the bazaars of district in Sivas center, Turkey. Mikrobiyol. Bull., 39(2): 219-223.
- Altındış, M., 2001. Besicilerinde in afyon, butchers, dairy products, collector and tasting in seropositive for brucellosis workers. J. İnfec., 15: 11-15.
- Anonymous, 1987. Commission of the European Communities. Code of good hygienic practices. EG-Documen: VI/5938/87 (PVET/2140).
- Anonymous, 2011. Ministry of Health. Retrieved from: <http://www.saglik.gov.tr/extras/dokuman/Data/genelbilgi.htm>.
- Arda, M., A. Minbay, N. Leloğlu and Ö. Akay, 1997. Special Microbiology-epidemiology: Bacterial and Mycotic Infections. Medisan Publication Series No. 26, Ankara, Turkey, pp: 197-223.
- Ataş, M., Ö. Poyraz, A. Alim, A.D. Ataş and A. Çelik, 2007. Offered for sale in Sivas in terms of fresh and pickled white cheese examination of brucella bacteria. Turk. J. Hygiene Exp. Biol., 64(2): 9-14.
- Bodur, H., N. Balaban and S. Aksaray, 2003. Biotypes and antimicrobial susceptibilities af *Brucella* isolates. Scand. J. Infect. Dis., 35: 337-338.
- De Buyser, M.L., B. Dufour, M. Maire and V. Lafarge, 2001. Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. Int. J. Food Microbiol., 67: 1-17.
- Erol, İ., 1997. Foodborne infections of public health importance in brucella. Production, 3(4): 33-37.
- Farrell, I.D., 1974. The development of a new selective medium for the isolation of brucella melitensis from contaminated sources. Res. Vet. Sci., 16: 280-286.

- Gaya, P., M. Medina, M.A. Rodriguez-Marin and M. Nunez, 1990. Accelerated ripening of ewes' milk Manchego cheese: The effect of elevated ripening temperatures. *J. Dairy Sci.*, 73: 26-32.
- Güllüce, M., A. Adıgüzel and Ö.F. Algur, 2003. Erzurum region various cheese samples provided by ELISA detection of antigens. *Turk. Mic. Cem. Derg.*, 33: 356-360.
- Kalender, H., Ö. Celal and N. Arslan, 2001. Fresh cheeses isolation of overalls. *Turk. J. Micr Harry*, 31(3-4): 184-186.
- Kaya, S., 2006. Problem and treatment of brucellosis. *J. Infec.*, 20(3): 227-230.
- Nielsen, K.H., L. Kelly, D. Gall, S. Balsevicius, J. Basse, P. Nicoletti and W. Kelly, 1996. Comparison of enzyme immunoassays for the diagnosis of bovine brucellosis (French). *Vet. Prev. Med.*, 26: 17-32.
- Patır, B. and A.H. Dinçoğlu, 2001. Fresh cheeses consumed in elazig research and tulum. *J. Vet. Med. Firat Univ., Milk. Sci.*, 15(1): 15-22.
- Ruben, B., J.D. Band, P. Wong and J. Colville, 1991. Person-to-person transmission of brucella melitensis. *Lancet*, 337: 14-15.
- Sancak, Y.C., B. Boynukara and H. Yardımcı, 1993. Van herbed cheeses brucella'ların a study on the existence and retaining time. *Veterinarium*, 4: 1.
- Sözen, T.H., 1996. Brucellosis. In: Artillery, A.V. and G. Doğanay (Eds.), *Infectious Diseases*. M. Nobel Medical Publishing House, İstanbul, pp: 486-491.
- Trujillo, I.Z., A.N. Zavala, J.G. Caceres and C.Q. Miranda, 1994. Brucellosis. *Infect. Dis. Clin. North Am.*, 8: 225-241.
- Trujillo, A.J., M. Buffaa, I. Casalsb, P. Ferná'ndezb and B. Guamisa, 2002. Proteolysis in goat cheese made from raw: Pasteurized or pressure-treated milk. *Innov. Food Sci. Em. Technol.*, 3: 309-319.
- Tunçbilek, M., 1992. Examination of fresh white cheese sold in the market in terms of the risk of brucellosis in Ankara. M.Sc. Thesis, Department of Food Hygiene and Technology, Institute of Health Sciences, Ankara University, Ankara, Turkey.
- West, H.G., 2008. Food fears and raw-milk cheese. *Appetite*, 51: 25-29.
- Yüce, A. and S.A. Çavuş, 2006. Brucellosis in Turkey: An overview. *J. Klim.*, 19(3): 87-97.