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Prevalence of Brucellosis in Sheep and Cattle Populations in a Rural Area of Western Anatolia, Turkey

[1]Recep Kara, [2]Zafer Çetinkaya, [3]İsmail Aytekin, [4]Levent Akkaya,
[1]Savaş Aslan, [5]Orhan Cem Aktepe and [4]Mukadderat Gökmen

[1]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine,
Afyon Kocatepe University, Afyonkarahisar, Turkey
[2]Department of Clinical Microbiology, Faculty of Medicine,
İstanbul Medeniyet University, İstanbul, Turkey
[3]Department of Internal Medicine, Faculty of Veterinary Medicine,
Balıkesir University, Balıkesir, Turkey
[4]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine,
Balikesir University, Balıkesir, Turkey
[5]Universal Ege Health Hospital, Konak, İzmir, Turkey

Abstract: *Background*: Brucellosis is considered to be the world's most common zoonosis that is particularly seen in the sheep, goats, cattle, buffalos and other farm animals. This study has aimed to evaluate the prevalence of the brucellosis in sheep and cattle in a Rural Area of Western Anatolia, (Turkey). *Methods*: In this scope, blood samples were obtained from 1065 sheep and 756 cattle while milk samples were obtained from 500 sheep and 500 cows in Afyonkarahisar provincial area. *Findings*: As a result, it has been detected that brucellosis positivity in sheep was13.9% with Rose Bengal (RB), 6.39% with Standard Tube Agglutination (STA); while the prevalence in cattle was 4.37% with RB, 3.56% with STA. It has been detected that brucellosis positivity in cow and sheep milk samples was 7.60% ve 5.40% with RB. In *Conclusion*: It has been specified that these ratios determined in Afyonkarahisar provincial area are over than the average of the results of the brucellosis tests performed by taking random samples from animals in the cities all over Turkey. Even though the struggle with Brucellosis is not at the aimed and expected level, some of the suggested measures to be taken to eradicate the infection are to separate sick animals from the herd, to avoid direct contact with the suspicious animals, to continue vaccination studies meticulously and to follow hygiene codes at all phases from production to consumption.

Key words: Brucellosis · Brucella Spp. · Blood · Milk · Rose Bengal · Standard Tube Agglutination

INTRODUCTION

Brucellosis is considered to be the world's most common zoonosis by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the International Office Epizooties (OIE) [1]. The disease is particularly seen in the sheep, goats, cattle, buffalos, canine, other farm animals and wild animals; yet, wild animals are reported to be the reservoir for the domestic animals [2]. *Brucella spp.* dwell in the organs like testicles and uterus resulting in abortion, loss of breeding value, infertility and the reduction in milk yield which cause losses in animal production and a public health problem with the direct contact with the infected animals and consumption of contaminated milk and dairy products [3]. The most commonly isolated ones are *B.abortus* and *B.melitensis* [4]. The infection in humans is called "Malta Fever" or "Floating Fire" [5]. Human brucellosis is commonly reported in the countries where people live in close contact with the animals, particularly

Corresponding Author: Recep Kara, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey. Tel: +902722281312, Fax: +902722281349.

in rural areas [6]. Brucellosis is a zoonotic disease which is widely seen in Turkey. The disease causes severe losses in manpower and material [7]. In spite of the ongoing vaccination program in Turkey since 1984, infection rate is very high in some provinces; the disease could not be adequately controlled [8]. This study was conducted to investigate the existence of *Brucella* spp. in the milk and blood samples obtained from dairy cattle and the sheep in a Rural Area of Western Anatolia, Turkey (Afyonkarahisar).

MATERIALS AND METHODS

In this study, blood samples from 756 cows and 2065 sheep and milk from 500 cows and 500 sheep were diagnosed. Blood and milk samples obtained from the cows and sheep, in Afyonkarahisar provincial area (Center, Suhut, Dinar, Isıklar, Cobanlar and Sinanpasa counties) were put into sterile tubes and transported to the laboratory under cold chain for analysis the same day.

Analysis of the Blood Samples: Rose Bengal Agglutination Test (RB): Blood samples that 50µl Rose-Bengal (RB) Agglutination Test Antigen (Spinreact, S.A., Sant Esteve De Bas, Spain) was added into 50µl blood serum on originally white chitin surface were stirred on mechanical rotator for 4 minutes.

Standard Tube Agglutination (Sta)test: Serums of blood samples have been taken; dilutions of blood serums have been prepared as 1:10, 1:20, 1:40, 1:80, 1:160 and 1:160< and 0.5 of Brucella tube antigen (Spinreact, S.A., Sant Esteve De Bas, Spain) for each has been added onto them and dilutions from 1:10 to 1:160 have been prepared. The tubes have been incubated at 37°C for 24 hours. 1:40 and above in serum antigen mixtures has been evaluated as positive. Positive samples at lower titers have been considered as suspicious.

Sheep and Cattle Milk Samples and Ring Test for the Milk: After the milk samples were kept in the fridge at least for 48-72 hours, tube agglutination was observed with the use of *B. abortus* S99 Ring test antigen (Vet-Vac, Veterinary Control and Research Institute, Pendik, İstanbul). 1 ml from each sample with 50µl of antigen was homogeneously mixed with milk vortex in the tube. The reaction occurring at the end of incubation at 37°C for 3 hours for the sheep milk and for 1 hour for the cattle milk was observed.

RESULTS

In this study a total number of 1821 blood samples from which 756 is from cows and 1065 from the sheep and 1000 milk samples of which 500 is from the cows and 500 from the sheep were collected from Afyonkarahisar provincial area. The blood sampled derived and tested with Rose Bengal (RB) test, 4.37% and 13.90% were positive for cows and sheep respectively. The serums which were identified as positive via RB test were examined with Wright Agglutination Test. That 1/40 and above were perceived as meaningful in serum agglutination test (Whey-AT), 3.56% of cow blood serums, 6.39% of sheep blood serums were identified as positive. Besides, 0.79% of cow blood serums and 7.51% of sheep blood serums which have agglutination below 1/40 titer were acknowledged as suspiciously positive (Table 1). 7.60% of cow milk and 5.40% of sheep milk tested with Rose Bengal Ring Test were identified as positive (Table 2).

DISCUSSION

Brucellosis has a worldwide distribution and remains a major problem in humans and animals in Middle Eastern and Mediterranean countries, where the prevalence is high. Brucellosis is also a health problem for humans and animals and causes economic loss due to the loss of animals [9]. Several studies have been conducted to determine the prevalence of brucellosis infection in cows and the sheep in Turkey. Esendal *et al.* [10] reported in their study on Brucellosis-suspicious cattle, sheep and goat serums that brucellosis seropositivity was 47.2% with RB and 51.6% with STA for the cattle; 37.6% with RB and 44.4% with STA for the sheep and goats. Ceylan *et al.* [11] reported Brucellosis seropositivity in their study as 19.6% with RB and 22.9% for the sheep and 20.9% with RB and 21.7 % with STA for the cattle. Kucukayan *et al.* [12] reported that 14.99% of the sheep which had abortion between 2003 and 2007 were identified to have brucellosis. Kucukayan *et al.* [12] found seropositivity with RB test as 2.67% for the cattle and 8.73% for the sheep in their study conducted around Kırıkkale province. They have reported that the all blood samples they identified as positive with RB test showed positivity with tube agglutination test as well. Sahin *et al*. [13] identified seropositivity in the North-east of Turkey at the rates of 35.30% with RB test and 32.92% with STA. Celebi and Atabay [14] discovered that Brucella seropositivity in the sheep from Kars provincial

Table 1: Brucellosis in Blood Samples of Cattle and Sheep

			Rose Bengal (%)	Titrations (%)					
	n	Positive	Negative	1/10	1/20	1/40	1/80	1/160	>1/160
Cattle	756	33 (4.37)	723 (95.63)	4 (0.53)	2 (0.26)	5 (0.66)	6 (0.79)	2 (0.26)	14 (1.85)
Sheep	1065	148 (13.90)	917 (86.10)	23 (2.16)	57 (5.35)	44 (4.13)	12 (1.13)	9 (0.85)	3 (0.28)
Total	1821	181	1640	27	59	49	18	11	17

n: No. of samples

Table 2: Brucellosis in Milk Samples of Cattle and Sheep

		Rose Bengal (%)	
	N	Pozitif	Negatif
Cattle	500	38 (7.60)	462 (92.40)
Sheep	500	27 (5.40)	473 (94.60)
Toplam	1000	65	935

n: No. of samples

area as 36.7% with RB and 35.5% with STA. Aras and Ates [15] determined seropositivity in the sheep as 41.1% with RB and 37.2% with STA in the study they conducted. In a study conducted by Sareyyupoglu *et al.* [16] brucellosis seropositivity in the cattle serum samples were detected as 3% with RB and 5% with STA. Brucella rates in cows' blood samples found in this study are lower than the studies conducted by Ceylan *et al.* [11], Apan *et al.* [9] and Sahin *et al.* [13] and higher than the findings of Sareyyupoglu *et al.* [16]. Brucella rates reported in this study in the sheep blood samples were lower than the ones of Ceylan *et al.* [11], Aras and Ates [15], Celebi and Atabay [14], higher than of Apan *et al.* [9].

The main cause of widespread infection among people, particularly in rural areas, is infected food, especially raw milk and dairy products [17-18]. In the studies conducted in various regions of Turkey on the sheep and cattle milk, the existence of Brucella has been investigated. Turutoglu *et al.* [19] found Brucella antibody positivity rates in cows' milk from Burdur area as 3% with Milk Ring Test, 2.2% with Whet-AT; 17.7% with Milk Ring Test nd 13.7% with Whey-AT for the sheep milk. Terzi [20] determined positivity in cow's milk in Samsun area as 20% with Milk Ring Test and 8% with Whey-AT. Brucella positivity rates determined for the cattle and sheep milk in this study were higher than the study of Turutoglu *et al.* [19] (2003) on the cattle milk and lower than the ones Terzi [20] obtained from their studies. Brucella positivity rates detected from the sheep milk were lower than the ones of Turutoglu *et al.* [19].

Comparing the results of tested milk and blood samples with the other studies, it can be stated that Brucella positivity rates in animals are at different levels. Despite ongoing eradication practices in some countries, these diversities in the studies in Turkey have been considered to result from mismanagement of the herds, taking unknown animals in the herd without adequate control, wrong vaccination practices, erroneous eradications and inadequate protective measures for animal health. The disease is associated with people's direct contact with animals, their discharges or secretions, consumption of dairy products, particularly fresh cheese, made of raw or unpasteurized milk and inhalation in the environment where sick animals are.

Preventing brucellosis, a zoonotic disease which issues a significant public health problem all around the world and in Turkey, is possible by close control of the animals and eradication of the infection. Not only breeders but also consumers of animal products are at risk. Hence, pasteurized milk should be consumed and used in the production of dairy products. At all stages of production and service from the farm to the consumer, sanitation should be enabled by the practices as HACCP (Hazard Analysis and Critical Control Points), GMP (Good Manufacturing Practices) and CCP (Critical Control Points), GHP (Good Hygiene Practices), OHSAS (Occupational Health and Safety). Precautions about thes ways of contamination, protection, health and hygiene should be considered by Medical Doctors and Veterinarians in the scope of One Medicine One Health.

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