Original Article

Prevalence of *Brucella* in Raw Milk: An Example from Turkey

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Abstract

Aim: Brucellosis is a highly contagious zoonotic infection affecting livestock and human beings. This study aimed to investigate the prevalence of *Brucella* in raw milk collected from a provincial center and central villages in the Central Anatolian region. **Materials and Methods:** This cross-sectional study was completed between March and September of 2016. The sample size for research was calculated as 263 milk samples with the Epi Info 2000 program. Samples were tested with the milk ring test, Rose Bengal test, and standard *Brucella* tube agglutination test. Suspicious samples according to these tests were seeded on medium for observation. **Results:** In this study, 202 cow's milk samples collected from 14 central villages were researched for the presence of *Brucella abortus*, a *Brucella* species bacterium. According to the medium seeding results, 35 of 202 raw cow's milk samples (17.32%) were identified as suspicious. **Conclusion:** The research investigated the prevalence of *Brucella* in milk samples collected from bovine farms used for consumption and production of raw milk products. The most significant infection route in our region is considered to be consumption of milk and milk products such as raw milk and fresh cheese. Especially in rural areas, households consuming their own produced milk are common. In regions with family-style milk and milk product production and consumption, interventional studies with the aim of improving knowledge, attitudes, and behavior related to zoonotic diseases should not be neglected.

Keywords: *Food safety, foodborne infection, milking hygiene, prevalence, zoonosis*

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Introduction

According to the World Health Organization (WHO), each year, millions of people become sick due to food-sourced zoonosis. One of the most significant bacterial zoonoses in terms of public health is the *Brucella* bacteria causing brucellosis.[1,2] Annual incidence is 0.3 cases per million in some developed countries, while in endemic regions, cases may reach >1000/million.[3] Although it has been eradicated in some developed countries, it is commonly observed in developing countries especially.[4,5] Even in countries where the disease is under control, studies on this topic continue intensely.[2,4]

Brucellosis is a disease that is difficult to report in both animals and humans.[6,7] According to the results of a research project run by the Ministry of Food, Agriculture and Livestock in Turkey, the prevalence of brucellosis in animals was identified as 1.43% and in sheep is 1.97%. This study, as with those in previous years, shows that the disease is widespread in Turkey in general, apart from in the Central and Eastern Black Sea, Aegean, and Mediterranean regions where animals do not migrate much and abortus cases are rare.[7]

One of the most important factors in the spread of *Brucella* infections among humans is milk and milk

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products. This is due to the form of consumption of milk and milk products which varies according to cultural habits and unhygienic, unhealthy factors in the preparation process of these products.[8-10] Many pathogenic organisms, such as *Brucella*, may remain viable if milk is raw or the boiling temperature is insufficient.[9] Together with the passage of bacteria into milk, in situations where good hygiene practices are not practiced the development of the disease in humans cannot be prevented. Sale of unpasteurized milk and use for long durations in the region of consumption have increased in recent years. Consumption of raw milk is not well documented; however, due to the current trend for "natural consumption" and "local sales," raw milk consumption has become popular. Although milk quality and safety is the topic of much research, the discussion about raw milk continues and this may be found primarily on the internet. In addition to scientific information, on the internet, generally, unscientific information is found. Considering the problems that may be encountered for each internet user in accessing accurate, healthy, and reliable information, the lack of control on bacterial content of raw milk forms a significant public health problem in terms of food safety and protecting health.[11-13] Literature investigation accessed limited information about pathogens sourced in milk and milk products in this region.

Farming and milk farming are intensely practiced in the research region, with incentives for investment in the area increasing. At the same time, there is insufficient field research into *Brucella* in the region. As a result, in this region with common and incentivized animal husbandry, monitoring milk production locations in terms of *Brucella* gains importance. In this study, the aim was to investigate the prevalence of *Brucella* in raw milk obtained from households earning their livelihoods from milk and milk products.

Materials and Methods

Sample collection

This cross-sectional study was completed in the Central Anatolian region in the months between March 2016 and September 2016. The population of the research comprised 9773 people living in the region. The population formula from the Epi Info 7 program (CDC, Atlanta, Georgia, USA) was used.[14] With expected prevalence of 50%, deviation of 5%, and 95% confidence interval, the sample size was calculated as 263 samples. For each village chosen for sampling, milk samples were obtained in proportion to the population. Milk samples were collected from villagers with their own animals living in the provincial center and central villages. From villages linked to the provincial center, a total of 202 (74.82%) cow's milk samples were taken [Table 1]. Milk samples were taken in sterile 50 ml capped tubes. While collecting milk samples, the basis was the number of cows on the farm and milk taken from each cow was placed in a separate sterile tube. Samples were placed in ice bags, preserving the cold chain and brought to the laboratory on the same day. To prevent cow's milk samples from giving a false-positive reaction to the milk ring test (MRT), they were left in a freezer for 24–48 h before analysis. *Brucella* MRT and tube agglutination test antigens, positive and negative control serums, and A and M nonspecific antiserums were obtained from İstanbul Pendik Veterinary Control Institute.

Microbiological analysis

Milk ring test

Milk samples placed in sterile 5 ml test tubes had one drop (0.03 ml) of *Brucella* MRT antigen dropped in and then were mixed. Tubes were incubated at 37°C for 1 h and results were evaluated 1 h later. If the cream on top of the milk in the tube was a different color to the milk and a definite ring was observed between the two, the reaction was assessed as positive.

Rose Bengal Test

Vet-Vac brand commercial Brucella Rose Bengal Plate Test antigen was prepared from Brucella abortus S99 strain, standardized with Brucella antiserum, and stained with Rose-Bengal. The antigen was left at room temperature for 15 min before use and shaken well. On a clean plate, 0.05 ml milk serum was dropped. Then, 0.05 ml Rose Bengal Lam Test antigen was added to this. The antigen and milk serum were mixed and spread over an area with diameter 1.5 cm. The plate was left in the air for 2 min by turning by hand. Formation of clusters of coarse particles was assessed as positive while homogeneous appearance was assessed as negative. Samples with positive Rose Bengal test (RBT), also called the diagnostic card test, had the Brucella standard tube agglutination test (STA) applied.

Brucella tube agglutination test

STA test was applied to samples with positive RBT and for this aim, the Vet-Vac brand commercial Brucella tube agglutination antigen was used. Tubes were prepared with serum dilution of 1/10, 1/20, 1/40, 1/80, 1/160, and 1/320. All tubes had 0.5 ml standard

Brucella tube agglutination antigen added. Thus, dilutions from 1/20 to 1/640 were obtained. The tubes were mixed and left at 37°C for 24 h to incubate. After incubation, clarity of the fluid in the tube with agglutination at the base of the tube was evaluated as a positive result. Tubes assessed as positive had titrations of 1/40 and above. To ensure better reliability of our results, samples with contradictory MRT and Brucella STA test results were seeded on Brucella agar medium.

Brucella agar (Fluka analytical 18795)

Samples seeded on medium generally begin to produce *Brucella* bacteria on the 3rd day at 37°C in an incubator, and highest proliferation is completed in 5–7 days. At the end of this period results, were evaluated.

Results

In this study, 202 cow's milk samples collected from 14 central villages were researched for the presence of *B. abortus*, a *Brucella* species bacterium. MRT was applied to all collected samples, and 69 of the 202 cow's milk samples (34.15%) had positive MRT results and 69 of the 202 cow's milk samples (34.15%) had positive MRT results. Similarly, all samples had RBT or diagnostic card test, applied after MRT. Of 133 samples negative on MRT, 2 were positive on RBT. Of the 69 samples positive on MRT, 33 were negative on RBT, with 38 of the 202 cow's milk samples (18.81%) identified as positive on RBT.

Samples positive on RBT had STA test performed. The 38 milk samples positive on RBT were positive on STA test at titrations of 1/40 and above. In conclusion, 25 of the 35 samples had antigen titration determined at 1/40 while 10 samples were identified at titration of 1/60. Twenty-two suspicious samples positive on MRT and negative on RBT or in the opposite situation were studied with STA test. Of these 22 samples, 11 remained at titration 1/20 while 11 had titration found at 1/10 [Table 2].

In our study, to investigate the reliability of contradictory milk sample results on MRT, RBT, and STA test, medium seeding was performed. Seventy-four (36.63%) milk samples with positive results on any of the previous tests were seeded on medium. Of the seeded milk samples, 35 (62.5%) displayed full proliferation providing results with the same value as the STA test.

When the tests are investigated, the results of 202 milk samples assessed 35 samples (17.32%) as suspicious.

Table 1: Numbers and test results of milk samples

Center/neighborhood/village	Number of samples collected	Number of positive serological tests (%)			
		MRT	RBT	STA	Medium
Center	64	28 (43.8)	11 (17.2)	11 (17.2)	11 (17.2)
Beşevler neighborhood	7	3 (42.9)	3 (42.9)	3 (42.9)	3 (42.9)
Kındam neighborhood	15	6 (42.9)	2 (14.3)	2 (14.3)	2 (14.3)
Gölhisar neighborhood	14	5 (33.3)	4 (26.7)	4 (26.7)	4 (26.7)
Sevdiğin village	6	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)
Çayağzı village	2	-	-	-	-
Yeşilli village	6	2 (33.3)	2 (33.3)	2 (33.3)	2 (33.3)
Çuğun village	10	3 (30.0)	2 (20.0)	1 (10.0)	1 (10.0)
Taburoğlu village	19	3 (15.8)	2 (10.5)	2 (10.5)	2 (10.5)
Toklumen village	6	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)
Karaduraklı village	4	-	-	-	-
Kumarkaç village	5	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)
Sıdıklı village	4	-	-	-	-
Uzunali village	6	4 (66.7)	3 (50.0)	2 (33.3)	2 (33.3)
Tosunburnu village	2	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
Dulkadirli village	7	4 (66.7)	1 (16.7)	1 (16.7)	1 (16.7)
Hamurluüçler village	15	2 (12.5)	2 (12.5)	1 (6.3)	1 (6.3)
Hashüyük village	10	3 (30.0)	2 (20.0)	2 (20.0)	2 (20.0)
Total	202	69 (34.2)	38 (18.8)	35 (17.3)	35 (17.3)

MRT=Milk ring test; RBT=Rose Bengal test; STA=Standard *Brucella* tube test

Table 2: Titration results of standard *Brucella* tube agglutination test

STA titration	*n* (%)
1/10	11 (19.29)
1/20	11 (19.29)
1/40	25 (43.85)
1/60	10 (17.54)
Total	57 (100)

STA=Standard *Brucella* tube agglutination test

Discussion

Brucellosis continues to be a problem in many regions in the world, led by developing countries especially.[15] According to the WHO data, the human incidence of brucellosis in Turkey is 262.2/million. Human cases occur linked to direct infection due to contact with carrier animals or consumption of raw milk and milk products obtained from these animals.[13] An endemic disease in Turkey, brucellosis, is more common in those who consume raw or not well-cooked milk and milk products, especially those living in rural areas, and these individuals comprise a potential risk group for the development of this disease.[16]

In this research, the prevalence of *Brucella* was investigated in milk samples collected from bovine animals on farms that consume and produce raw milk and milk products. The collected milk samples had MRT, RBT, and STA test performed, respectively, with medium seeding of samples with suspicious results on the three tests. Linked to the results of the three tests, when medium seeding results are assessed, 35 of the 202 raw cow's milk samples (17.32%) were identified as suspicious.

Seroprevalence studies in different regions identified the *Brucella* prevalence in cow's milk samples as 34.78% in Kars and 10.37% in Kayseri.[17,18] A study of humans, sheep, and cattle in Kirikkale province by Apan *et al*. identified the seropositivity in animals as 6.47%.[19] In our study, the seropositivity was identified as 17.32% and was close to the seropositivity in Kayseri. Kirikkale, Kayseri, and Kirşehir provinces are located in the same region (Central Anatolian region) and are neighboring provinces. Beef farming and milk farming are practiced in these provinces, with common cultural properties involved in the production and consumption routes of milk and milk products and hygienic behavior in preparation by the locals. Due to these similarities, it is considered that the *Brucella* seropositivity is similar in these provinces. A study in another region (Aegean region) by Kenar and Altindiş found that 6 of 120 milk samples collected in the Afyon region were positive on agglutination and ring test for a 5% rate of *Brucella* antibodies.[20] This rate is lower than our findings and other results from our region which is probably due to both production and consumption habits, as well as animal movement and controls, being different between regions. Other social determinants affecting health such as education and socioeconomic level in the Aegean region may be different compared to other regions which are considered to affect hygienic behavior. However, our study has some limitations. First, we did not evaluated knowledge, attitudes, and behaviors of participants on safety milk production. Therefore, we could not discuss the risk factors that may affect the prevalence.

Public health experts play an important role in the prevention of diseases linked to zoonosis and in the protection of public health. With this aim, the WHO recommends that surveillance systems for food-sourced diseases should be strengthened and that data obtained from this surveillance be used in the planning, operation, and assessment of public health policies.[21]

Conclusion

Given that the prevalence of *Brucella* in raw milk in the Central Anatolian region is changed between 5% and 17%,[18-20,22] according to our results (17.32%), it seems necessary to control this disease. In the region, brucellosis in humans and animals appears to form a significant public health and veterinary health problem. It appears that projects and studies to protect against brucellosis should focus on hygienic production and consumption of milk and milk products especially. Care should be taken during inspections in the stages of production and consumption.

The first stage in controlling zoonotic diseases is periodic observation of the prevalence and distribution of the diseases in animals, making timely intervention against epidemics, vaccination, and prevention of the disease in animals possible. As a result, the primary and significant public health and veterinary health problem of brucellosis leads the list of zoonotic character infectious diseases in Turkey. Regular collection of milk samples to monitor the prevalence and incidence of the disease in animals should research *Brucella* species and intervention planning is necessary.

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Conflicts of interest

There are no conflicts of interest.

References

1. Pieracci EG, Hall AJ, Gharpure R, Haile A, Walelign E, Deressa A, *et al*. Prioritizing zoonotic diseases in Ethiopia using a one health approach. One Health 2016;2:131-5.
2. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis 2006;6:91-9.

3. Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, Paul M, *et al.* Treatment of human brucellosis: Systematic review and meta-analysis of randomised controlled trials. BMJ 2008;336:701-4.
4. European Food Safety Authority, European Centre for Disease Prevention, Control. The European union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J 2015;13:4329-35.
5. European Food Safety Authority, European Centre for Disease Prevention, Control. The European union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. EFSA J 2017;15:4694-9.
6. Yumuk Z, O'Callaghan D. Brucellosis in turkey -- An overview. Int J Infect Dis 2012;16:e228-35.
7. Zencir M. Occupational Infectious Diseases: Health of Healthcare Workers Example. Definition of Occupational Diseases ; 2015.
8. Sam IC, Karunakaran R, Kamarulzaman A, Ponnampalavanar S, Syed Omar SF, Ng KP, *et al.* A large exposure to *Brucella* melitensis in a diagnostic laboratory. J Hosp Infect 2012;80:321-5.
9. Chye FY, Abdullah A, Ayob MK. Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol 2004;21:535-41.
10. Taiwan CDC Confirms this year's Fourth Imported Case of Brucellosis and Urges Public to pay Attention to Dietary Hygiene while Traveling Overseas to Prevent Brucellosis Infection. CDC; 15 September, 2011.
11. Barbano DM, Ma Y, Santos MV. Influence of raw milk quality on fluid milk shelf life. J Dairy Sci 2006;89 Suppl 1:E15-9.
12. Elmagli A, Ibtisam E, Zubeir E. Study on the hygienic quality of pasteurized milk in Khartoum State (Sudan). Res J Anim Vet Sci 2006;1:12-7.
13. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: A re-emerging zoonosis. Vet Microbiol 2010;140:392-8.
14. CDC. Epi Info 2017. [updated 2017 Sep 13; cited 2018 Jan 20] Available from: https://www.cdc.gov/ epiinfo/index.html.
15. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. Lancet Infect Dis 2007;7:775-86.
16. Ozcan H, Sahin M. Investigating the level of knowledge about brucella disease, according to the size of the farms. Gümüşhane University J of Health Sciences: 2012;1:211-224.
17. Sahin M, Genç O, Unver A, Otlu S. Investigation of bovine brucellosis in the northeastern turkey. Trop Anim Health Prod 2008;40:281-6.
18. Inci A, Aydin N, Babur C, Cam Y, Akdogan C, Kuzan S. Seroepidemiologic investigations on toxoplasmosis and brucellosis in bovine and sheep in Kayseri region. Pendik Veteriner Mikrobiyol Derg 1999;30:41-6.
19. Apan TZ, Yildirim M, İstanbulluoğlu E. Seroprevalence of brucellosis in human, sheep, and cattle populations in Kırıkkale (Turkey). Turk J Vet Anim Sci 2007;31:75-8.
20. Kenar B, Altındiş M. An investigation of *Brucella* antibodies in milk samples collected from Afyon region. Turk Hij Den Biyol Derg 2001;58:87-92.
21. WHO. Zoonosis, [Veterinary Public Health; 2017. [updated 2018; cited 2018 Jan 20] Available from: http://www.who.int/zoonoses/vph/en/.
22. Serttaş B. Detection of *Brucella abortus* Antibodies in Cow's Milk of the Isparta Area by Ring and ELISA 2006. PhD Thesis. Institute of Science, Süleyman Demirel University; 2006.