



Original Article

Prevalence of *Brucella Species* in Raw Milk Produced in the Industrial and Traditional Production Units in Yazd

Farideh Jamali¹ M.Sc., Sahar-sadat Emadi² M.Sc.,
Ahmad Mosadegh^{2*} M.Sc.

¹Department of Biology, Islamic Azad University, Ashkezar Branch, Iran.

²Department of Microbiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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Key words

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Backgrounds and Aims: Healthy milk and dairy products are important in human nutrition because of the high value of the food. Contaminated raw milk is the mainly way of transmission of brucellosis to humans. Unpasteurized milk and dairy products can contaminate to human and cause malta fever. So brucellosis is an important zoonotic disease in human and animals especially domestic animals. The aim of this study is determining the contamination of traditional and industrial cow and sheep milk to *brucella* spp in Yazd.

Materials and Methods: Totally 198 raw milk samples were randomly cultured in three months period from the units of distribution supply of the milk centers at different regions in Yazd, examined by special *brucella* agar, species identification was performed by Gram stain, and growth in the presence of aniline dyes, hydrogen sulfide production, and urease test.

Results: Five samples (2.52%) were positive, 4 samples (2.02%) of *Brucella.abortus* and 1 sample (0.5%) of *Brucella.melitensis* were confirmed out of 198 cultured milk samples.

Conclusions: The results show the seriousness of the brucellosis transmission to human (Malta fever) through raw and unpasteurized milk.

*Corresponding Author: Department of Microbiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Tel:+989131527495, Email: mosadegh14@yahoo.com

Introduction

The health of milk and dairy products are highly important for the consumers. Thousands of people worldwide are infected with bacteria through milk and dairy products each year that cause infection and food poisoning. So milk is the most contaminated dairy product with 77.1% [1]. There is a wide variety of bacteria in milk, that one of them is *brusella* [2]. *Brucella* is a small gram-negative bacterium, facultative intracellular, highly aerobic and fastidious, which cause brucellosis in cows, sheep, goats and humans (zoonotic disease in humans and animals) [3]. *Brucella* is classified to six species based on the difference between the main host and pathogen: *Brucella canis*, *neotome*, *ovis*, *suis*, *abortus*, and *melitensis*. *Brucella abortus* is a bovine brucellosis that causes Raging fever (brucellosis) in humans. Truly this disease is also caused by *Brucella melitensis*, *Brucella canis* and *Brucella suis* [4]. Brucellosis is the cause of infertility and miscarriages in animals and also the cause of Raging fever, meningitis, arthritis, and endocarditis in humans. Most of human brucellosis are related to *Brucella abortus* and *Brucella melitensis*.

The most common way of transmission of infection to human is done through the mucosa (droplets), respiration, skin (contact with infected animal tissues), particularly gastrointestinal (eating contaminated milk and dairy products) which is determined by fever, chills, sweating, urinary tract obstruction, liver complications, joint pain, headaches, and

general pain [5, 6]. Brucellosis is still one of the most common infections in developing and even in some developed countries.

Nowadays the control of brucellosis is based on three options: killing of infected animals, pasteurization, and vaccination. Animals' vaccination is based on the alive attenuated inoculation strains of *Brucella abortus* S19 and *Brucella melitensis* Rev1. Using of this vaccine has been restricted in animals. The stimulation of the production of antibodies in vaccinated animals prevents separating them from affected animals. Also, abortion and brucellosis disease in these animals have caused inoculation of vaccine forbidden in humans. Animals are prohibited to be taken with caution [7].

There is a hyper endemic brucellosis in Iran. As brucellosis in cows has often no clear clinical symptom, and often does not cause any changes in the appearance of the milk; so the infection may permanently be present in infected animals and causes secretion of *Brucella* from animal's milk [8]. Approximately twenty-three thousand new cases of brucellosis are reported in Iran each year that in most parts of Iran are widespread in humans and animals; so checking and careful planning for controlling and eradication is necessary [9].

Materials and methods

In this study, 198 samples of raw milk were collected from industrial factory, dairies countryside, traditional dairy product, and

distribution units; that 148 is cow's milk and 50 is sheep's milk. The samples were collected randomly and in sterile containers, immediately were transferred to the laboratory full profiled, and were tested. The Ethics Committee of Islamic Azad University, Ashkezar branch, confirmed this project.

Preparation of *Brucella* agar medium (Merck, Germany): This is a basic culture medium for the growth and isolation of some bacteria, such as *Brucella* spp. This is made of a basic medium such as tryptone with the origin of protein, sodium chloride, agar, and 5% blood. As the *Brucella* is a fastidious bacterium and has complex nutritional needs; it should add a certain amount of antibiotics to the cultured medium for preventing the growth of other bacteria. So we can reach the selective medium [10].

First step: at first, the samples were quite homogeny and kept in refrigerator for an hour. Pour 5 ml of each milk sample in tube at sterile condition and centrifuged for 10 minutes at 3500 rpm. So, the cream (the upper, fat) and protein deposition (milk) were observed in the tube. The cream was cultured linearly in the *brucella* agar medium. Then milk was cultured after cream.

Second step: put the plates which were cultured in anaerobic jar because of the CO₂ production. It will incubate at a temperature of 35-37°C for 48 hours.

Third step: Confirmatory tests are done on the culture medium that has colonies shapes, colors, special consistency (small colonies white to gray, non-mucoid colonies), and is separated from other samples. So, *Brucella*

species are clear, convex, flat with straight edges, and has 1-2 mm diameter.

Considering the slow growth of *Brucella*, they were kept up to 1 week for ensuring the negative sample of *brucella*. Identification species were done by gram stain, growth in the presence of color, hydrogen sulfide production, and urease test, which are as follows:

Gram stain: Safranin is on the samples for 3 minutes and *brucella* has been seen light red

Urease test: All species except *Brucella ovis* and some strains of *Brucella. melitensis* are positive.

Hydrogen sulfide production: *Brucella abortus* involves H₂S production for at least 4 days, but *Brucella.melitensis* does not involve [10].

Growth in the presence of color: fuchsine and thionin colors were added by filtering *brucella* agar medium without antibiotics. Color concentration should be:1:50,000, incubate the plates for 4 days in the presence of CO₂ after culturing.

Results

In this study 198 raw milk samples are collected in three months period from distribution and supply of milk centers at different regions of Yazd. According to results 139 (70.21%) traditional cow's milk, 9 (4.54%) industrial cow's milk, and 50 (25.25%) sheep's milk, out of 198 samples, were our samples. We have not got any industrial sample for sheep's milk (Table 1).

Table 1. Absolute and relative frequency of collected milk

Milk prepared	Cow		Sheep		Total	
	Number	Percent	Number	Percent	Number	Percent
Traditional	139	70.21%	50	25.25%	189	96.5%
Industrial	9	4.54%	0	0%	9	4.54%
Total	148	74.75%	50	25.25%	198	100

184 samples (92.93%) which were collected in the traditional method and 9 (4.54%) which were collected in the industrial method, were reported negative and 5 samples (2.52%) prepared in the traditional

way were positive after culturing and identification. According to the observations reported, we do not have any positive industrial sample. The results are shown in table 2.

Table 2. Absolute and relative frequency of positive and negative samples in collected milk

Milk prepared	Negative sample		Positive sample		Total samples	
	Number	Percent	Number	Percent	Number	Percent
Traditional	184	92.93%	5	2.52%	189	95.45%
Industrial	9	4.54%	0	0%	9	4.54%
Total	193	97.48%	5	2.52%	198	100

Brucella spp. was identified after separation of positive samples and confirmation tests as follows: 4 (80%) samples of cow's milk

contaminated to *Brucella abortus*, and 1 sample (20%) of sheep's milk to *Brucella melitensis*. (Table 3).

Table 3. Absolute and relative frequency *brucella spp.* in collected cow and sheep milk

Milk prepared	<i>Melitensis species</i>		<i>Abortus species</i>		Total	
	Number	Percent	Number	Percent	Number	Percent
Cow	0	0%	4	80%	4	80%
Sheep	1	20%	0	0%	1	20%
	1	20%	4	80%	5	100
Total						

Identification species in positive samples has shown 1 (0.5%) *Brucella melitensis* sample and 4 (2.02%) *Brucella abortus* among the total samples in traditional milk, and as

mentioned it was not a positive sample of industrial milk. The results are presented in table 4.

Table 4. Absolute and relative frequency *brucella spp* in traditional and industrial collected milk

Milk prepared	<i>melitensis</i> species		<i>Abortus</i> species		Total	
	Number	Percent	Number	Percent	number	Percent
Traditional	1	0.5%	4	2.02%	5	2.52%
Industrial	0	0%	0	0%	0	0
Total	1	0.5%	4	2.02%	5	2.52%

Discussion

Brucellosis is one of the most important diseases between humans and animals that are transmitted to humans by eating contaminated animal products. Unpasteurized dairy products in areas contaminated by brucellosis are one of the most important ways of transmission of Malta fever to human. They are highly variable and nonspecific clinical symptoms and can occur from a febrile acute illness to a mild and uncertain illness [11]. The global spread of the brucellosis is still considered as the serious health problems in humans and domestic animals. Although the published statistics are different incidences of the disease in several countries and their prevalence is not clear, but statistics published between 0.01 to 200 cases and 132.4 cases per 100,000 people in Iran [12].

All species of *Brucella* that are important for pathogenicity in humans can be disposed through the milk of infected animals. Since the distribution of brucellosis is much higher

compared to the other infections in animals and humans due to many different ways of secretion of *Brucella*, and infected animals remain carriers for long periods; anything that causes transmission of the disease has a great importance. Therefore the contamination of the milk to brucellosis is important. So, investigation must be carried out in the field of development, and disease control that determine the contamination of raw milk and other dairy products is a priority [2].

Ismaili and partners reported sheep's and goat's milk contamination 5.3 percent, and 2.9 percent for cow's milk *Brucella* in three provinces of Iran. They sampled randomly from different herds in their study [13].

Mohsenzadeh and partners have done a study titled prevalence of *Brucella* in raw milk supply to the market and its effect on public health in 1382, in Mashhad. The results have showed that 6 samples were positive and 2

cases were confirmed *Brucella abortus* out of all the samples [14].

Ilhan and partners (1999) showed that 4 (8%) cases were infected to *Brucella abortus* [8] during a study of 50 samples of cow raw milk. Mert (1984) did a study on 150 samples of white Turkish cheese prepared from raw milk at Ankara. The results have showed that 19.3% of the samples were *Brucella* infected in which 90% cases contain *melitensis* and *Brucella abortus* was isolated in 10% of cases [15].

The high level of contamination of dairy products in the studied areas indicated high prevalence of the bacteria in the animal population in those areas. Every year a significant number of people may have Malta fever in countries and areas where the consumption of raw and unpasteurized milk and dairy products is common, due to consumption of such nutrients.

In this study, the contamination of samples tested for *Brucella* was totally determined 2.52% which is less than some other studies. A large number of samples in this study are compared to the samples of other studies as the main features of this plan. Industrial samples were not found positive. 2.02% were *Brucella abortus* that can cause further supply raw milk compared to goat's and sheep's raw milk. In previous studies, most species of *Brucella abortus* were isolated, but in the present study the species of *Brucella abortus* and *Brucella melitensis* were identified. The major problem

of Brucellosis in Iran is *Brucella melitensis* that is caused by species of goats and sheep. Goat's milk is even tested in addition to cow's milk in this study, but other studies have used only cow's milk. It is better to vaccinate cows against diseases associated with teaching pasteurized role in preventing the spread of brucellosis to villagers, considering that sheep's milk is not placed in country's pasteurization cycle, and Dairy products are traditionally produced in rural areas.

Conclusion

This study has been done for the first time in Yazd. Relatively low levels of contamination of dairy products to *Brucella* are shown in this province according to the results and as compare them to the studies done in other provinces. The necessity of prevention is very important according to the past history of the brucellosis in the Middle East and especially in Iran, including animal and human brucellosis. Controlling brucellosis in each country identification according to species should be first done on the agenda in labs.

Conflict of interest

The authors declare that there are no conflicts of interest associated with this manuscript.

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