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Brucellosis in Humans with the Approach of *Brucella Species* Contamination in Unpasteurized Milk and Dairy Products from Hamadan, Iran

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ABSTRACT

Background and Aim: As the most important human food source, milk and dairy products may lead to infectious diseases due to non-compliance with health standards. Brucellosis is one of the critical zoonotic diseases that affect the human population. Humans are usually infected by *Brucella spp.* via contaminated milk and dairy products and direct contact with infected animals.

Materials and Methods: This study was conducted to determine the *Brucella spp.* contamination rate of milk and dairy products in the rural and urban areas in the city of Hamadan, west of Iran, in 2018-2019. In this descriptive-analytical study, 291 samples of nonboiling milk (227), fresh cheese (43), and cream (21) were collected from dairy products suppliers in the urban (No=103), rural areas (No=162), and industrial regions (No=26). We collected 72 samples from sheep and goats and 219 specimens from cattle. Samples were randomly selected from the target centers.

Results: The overall contamination rate of collected samples with *Brucella spp.* found to be 4.1%. The milk and dairy products contamination in urban areas was 0.9%, rural 6.6%, and industrial regions 0%. Furthermore, the contamination rate varied from 9.7% to 2.5% for small ruminants and large ruminants, respectively, which was significant (*P*=0.01).

Conclusion: Given the importance of dairy consumption in the human diet and higher contamination of milk and dairy products taken from cattle, sheep, and goats with Brucella species, it is recommended that control and prevention programs in sheep and goats must be taken more seriously.

Keywords: *Brucella*; Contamination; Dairy products; Hamadan

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1 Introduction

Brucellosis is an important zoonosis with global distribution which can happen in 3 stages: acute, subacute, or chronic **(1, 2)**. The causes of brucellosis are small, gram-negative, non-motile, and facultative intracellular coccobacillus, which can infect many mammals, including cows, sheep, goats, pigs, rodents, marine mammals, and humans **(3)**. Brucellosis can affect the reproductive system and lessen fertility or even cause spontaneous abortion or infertility, especially in cattle **(1, 4)**. The humans are infected by *Brucella spp.* via the gastrointestinal tract, respiratory system, and non-intact skin **(5, 6)**. The Bacteria disseminate in the body through the blood circulation and lymphatic system **(7)**. Brucellosis usually presents fever, sweating, weakness, musculoskeletal pain, lethargy, and weight loss. Also, it can cause local

infections such as meningitis, hepatitis, orchitis, and some other local involvements **(8)**. Six species of Brucella have been identified as disease-causing agents, which are *Brucella abortus*, *Brucella canis*, *Brucella melitensis*, and *Brucella suis,* capable of causing disease in humans **(2)**. Humans are usually infected by consuming contaminated milk and dairy products **(9)**. Brucellosis may transmit through non-intact skin, even the placenta, and also it is an airborne disease **(10, 11)**. Other raw or semi-cooked beef by-products such as liver, meat, heart, kidney, and blood, which are common foods in some countries, are considered to be infectious sources **(12)**.

Brucellosis is a zoonosis disease that can spread between animals and humans worldwide, especially in the Mediterranean countries, the Middle East, the Arabian Peninsula, Central, and South America, Asia, and Africa. Although only 17 countries such as Scandinavian and northern European countries, Australia, New Zealand, Japan, and a couple of other countries have been declared free of brucellosis, even in these countries, some cases of the disease have been reported among travelers to endemic areas **(13)**. The countries like Iran, Saudi Arabia, Syria, Jordan, and Oman have the highest incidence of human brucellosis, and the incidence of brucellosis in the Middle East is between 1 to 78 people per 100,000 populations **(14)**. According to the Lancet journal statistical data in 2006, Iran, Turkey, Iraq, and Saudi Arabia, with an outbreak of 8 to 50 per 100,000 after Syria (over 100 per 100,000 population), Afghanistan, Georgia, Bosnia, and Albania (50 to 100 per 100,000) has the highest prevalence of human brucellosis **(15)**. Otlu *et al.* in a study in Turkey showed that in some provinces of the country, 34.9% of the livestock with a history of abortion had positive brucellosis history. Brucellosis is endemic in Iran, Syria, and Iraq, especially in provinces neighboring Turkey, due to the illegal livestock exchange **(16)**. The disease is widespread in all parts of Iran. Still, its prevalence is not the same in different regions, so the least incidence reported in the southern regions of Iran and the highest infection rates (31 to 41 cases per 100,000 people) occur in the provinces of Hamadan, Markazi, East Azarbayjan, and Zanjan **(2)**. This study was designed and conducted to investigate the Brucella infection of milk and dairy products that have been used in different areas of Hamadan, west of Iran, in a local dispensary in unpasteurized form.

2.Materials and Methods

Sampling

This cross-sectional and descriptive-analytical study was performed on milk (227 samples), soft cheese (43 samples), and creams (21 samples) from traditional or non-pasteurized dairy supply centers in Hamadan, west of Iran (urban and rural areas), and industrial regions. Milk samples were collected from local unpasteurized dairy distributors in urban areas and industrial sites that passed all hygienic processes. Regarding cheese samples, half of them were collected from dairy product dispensaries with sources in villages, countries, and half-regular pasteurized cheese. The cream samples were taken from stores or rural houses prepared in a traditional and non-pasteurized manner. This study was approved by the ethics committee of Hamadan University of Medical Sciences (No: IRUMSHA. REC.1393.930222646).

Diagnosis and Identification of *Brucella spp.*

To detect Brucella spp. in samples, 100-200 mL milk was collected and centrifuged at 3000 rpm for 20 minutes, and the pellet was plated on *Brucella* agar medium supplemented with different antibiotics and incubated in a 10% CO_2 incubator at 35°C for at least seven days. One hundred grams of fresh cheese and cream were taken into sterilized tubes, homogenized in *Brucella* broth medium, and centrifuged at 3000 rpm for 20 minutes. The supernatant was discarded; then, 10 mL of *Brucella* broth medium was added to the pellet and incubated at 35°C for 24 hours. After the preliminary incubation by a sterile loop, inoculate the sample on a medium supplemented with different antibiotics. The following concentrations of antibiotics were added per liter of media to eliminate undesirable microorganisms: cycloheximide (100 mg), bacitracin (25000 units), polymyxin B sulfate (5000 units), vancomycin (20 mg), nalidixic acid (5 mg) and nystatin (100 000 units). The plates were then incubated in a 10% CO_2 incubator in a humidified atmosphere at 35°C for at least seven days. On culture, colonies appear small, convex, smooth, translucent, nonhemolytic, and slightly yellow and opalescent after at least 48 hours of incubation, and suspected colonies were identified by gram staining, CO_2 requirement, and biochemical tests such as H2S production, susceptibility to the aniline dyes thionine and basic fuchsin, urease, oxidase, and catalase **(17, 18)**.

Data Analysis

The obtained data and values are entered into SPSS software 20 (SPSS Inc., Chicago, IL., USA), and the results were analyzed using descriptive statistics by performing Fischer's exact test.

3.Results

Out of 291 samples, 12 (4.1%) samples showed Brucella growth, and after identification and confirmation tests, 9 (75%) samples were found to be *B. melitensis,* and the remaining 3 (25%) were *B.*

abortus (Tables 1, 2, and 3). The Brucella contamination rates in urban, rural, and industrial regions were found to be 2(1.9%), 10 (6.2%), and 0.0%, respectively (Table 1). Fischer's exact tests did not show significant differences in urban and rural areas (*P*=0.186). Of the 103 samples collected from urban areas, two were positive for Brucella infection, one being *B. melitensis* and the other *B. abortus*. On the other hand, out of 162 samples collected from rural areas, ten samples showed bacterial growth, which was found to be 8 cases of *B. melitensis* and 2 cases of *B. abortus*. The contamination rates samples were 4%, 2.3%, and 9.5% in milk, cheese, and cream, respectively. Fisher's exact test did not show any significant difference in the three samples (*P*=0.332) (Table 2).

Table 1. *Brucella spp.* dairy product contamination rate based on sampling sites

Area	Positive No(%)	Negative No(%)	Total No(%)
Urban	2(1.9)	101(98.1)	103(100)
Rural	10(6.2)	152(93.8)	162(100)
Commercial	0	26(100(	26(100)
Total	12(4.1)	279(95.9)	291(100)

Table 2. *Brucella spp.* contamination rate in the dairy product based on the type of samples

Sample	Positive No(%)	Negative No(%)	Total No(%)
Milk	9(4)	218(96)	227(100)
Cheese	1(2.3)	42(97.7)	43(100)
Cream	2(9.5)	19(90.5)	21(100)
Total: No(%)	12(4.1)	279(95.9)	291(100)

The contamination rate of dairy samples by type of livestock (sheep and cow) was shown in Table 3. Of the dairy samples obtained from cows and sheep, respectively, 2.3% and 9.7% were contaminated with Brucella spp. Fisher's exact test showed a significant difference in the source of products (*P*=0.012), which means that the outbreak of brucellosis in sheep is more than the cow.

Table 3. *Brucella spp.*contamination rate in the dairy product based on the type of livestock

livestock	Positive No(%)	Negative N (%)	Total No(%)
Sheep	7(9.7)	65(90.3)	72(100)
Cow	5(2.3)	214(97.7)	219(100)
Total :No (%)	12(4.1)	279(95.6)	291(100)

4.Discussion

The use of unpasteurized dairy products such as milk, cheese, cream, and whey is the source of some infections and diseases in humans. These diseases are classified as foodborne diseases and are mainly caused by bacteria such as *Listeria monocytogenes*, *Salmonella spp., Escherichia coli, Staphylococcus aureus, Bacillus cereus, and Campylobacter spp.* **(17)**. In addition to being a zoonosis disease between humans and livestock, brucellosis is also transmitted through milk and dairy products **(19, 20)**. In the present study, 12 samples (4.1%) of dairy products, including milk, cheese, and cream infected by Brucella spp. In a study by Bateni *et al.* in Zanjan, out of 299 milk and cheese samples examined by culture method, 5 samples (1.67%) were infected by Brucella **(21)**. In other research reported by Movasagh *et al.* in the Parsabad-Moghan region of Ardabil province, Iran, the raw cattle milk contamination rate was 37.5% **(22)**. In another study carried out by Akbarmehr *et al.* conducted on 1000 samples of cheese from Sarab city

in Iran and its suburbs from 1999 to 2001, they found Brucella infection rate to be 2.2 %, which 0.7% and 1.5% of the cases were reported as *B. melitensis* and *B. abortus* respectively **(23)**. Izadi *et al.* investigated the rate of Brucella infection in milk and dairy products using the Nested PCR technique in Tehran province, Iran. In 34 pasteurized milk samples, they reported 10 cases were PCR positive, from 28 pasteurized cheese samples, only 8 cases, from 23 traditional cheese 14 cases, and finally, from 33 samples of raw sheep milk, 19 cases were PCR positive **(24)**. It seems that the differences between the results of the current study and previous ones are related to the methodology and the area of research **(25, 26)**.

A study on 1028 brucellosis patients in Turkey showed that 63.6% had a history of raw dairy products and/or raw milk consumption. In Turkish studies, the level of reported human Brucella contaminations resulting from infected dairy products varies from 62.6% to 94.6%. Infected raw milk consumption was also accountable for 69% of brucellosis cases in Kuwait, 57.1% in Iran, and 63% in Oman. Recently in Qatar, an outbreak of *B. melitensis* and *B. abortus* infections has been accompanied by camel milk drinking. Eating unpasteurized raw milk and cheese has also been reported as an important source of human brucellosis in other Middle Eastern areas such as Saudi Arabia **(27)**. Khalili *et al.* in Kerman city, Iran, reported that the rate of Brucella contamination in the delivery tank to one of the dairy factories by polymerase chain reaction (PCR) method was 3.8%, which is more than our findings **(28)**. Movasagh *et al.* took 50 random samples of cow's milk and, by using the ELISA method (Milk ring test), reported that 10% of samples were positive for *B. abortus* **(29)**. In a study by Silva *et al.* in Amazon areas on samples of cow's milk and cheese from buffalo milk, the Brucella infection rate was reported at 21% (14 samples out of 66), one of which was caused by the vaccine strain and in all of the other cases *B. abortus* isolated **(30)**. In Egypt, the study of Gamal Wareth showed that from 215 bovine milk and milk products using indirect enzyme-linked immunosorbent assay (iELISA), anti-Brucella antibodies were detected in 34 samples (16%).

In contrast, the real-time PCR (RT-PCR) technique amplified Brucella-specific DNA from 17 milk samples (7.9%), which 16 of the RT-PCR-positive samples containing *B. melitensis* DNA; 1 RT-PCR-positive sample was identified as having *B. abortus* DNA **(31)**. In another study in Turkey, fresh cheese from sheep milk, which was for sale in the central market of Cannakale, was investigated by Alper *et al.* by culture method, the rate of infection was found to be 0 % **(32)**. Considering the high level of contamination in dairy products, it seems that using boiled milk to prepare cheese might be an effective way to combat the disease **(33, 34)**.

5.Conclusion

This study showed that unpasteurized milk and dairy products are highly contaminated with Brucella spp. Therefore consumption of raw milk and unpasteurized dairy products is considered a serious public health hazard. The old belief about the usefulness of raw milk over pasteurized milk should be considered in light of current scientific information.

In general, education about the nature of the infection and how it is spread through raw milk and dairy products is essential to prevent infection or the spread of the disease.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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