The contamination rate of Brucella spp. in unpasteurized dairy products used in urban and rural areas of Hamadan County, west of Iran

Mohammad Mahdi Majzoobi
Brucellocis research center, Hamedan university of medical sciences, Hamedan, Iran
Pezhman Karami
Brucellocis research center, Hamedan university of medical sciences, Hamedan, Iran
Amir Khodavirdipour
St. John's hospital, Bangalore, India https://orcid.org/0000-0003-0404-3430
Mohammad yousef Alikhani (alikhani43@yahoo.com)
https://orcid.org/0000-0003-4577-4029

Research article

Keywords: Brucella, Culture, Contamination, Dairy products

Posted Date: February 5th, 2020

DOI: https://doi.org/10.21203/rs.2.22703/v1

Abstract

Background: Brucellosis is one of the most prevalent and important zoonotic diseases which is affecting the significant number of livestock and human population. Humans usually infected by *Brucella spp.* via contaminated milk and dairy products and direct contact with infected animals. This study was conducted to determine the *Brucella spp.* contamination of cottage dairy products in the rural and urban areas in the city of Hamadan, west of Iran.

Results: In this descriptive-analytical study, 291 samples of nonboiling milk (227), fresh cheese (43), and cream (21) were collected from dairy products suppliers at the city center (No=103), villages (No=162), and industrial parks (No=26). We collected 72 samples from sheep and goats and 219 specimens from cattle. Samples were randomly selected from the target centers. The overall contamination rate of collected samples with *Brucella spp.* found to be 4.1%. Contamination of milk and dairy product in urban areas was 0.9%, rural 6.6%, and dairy farms 0%. Furthermore, the contamination rate varied from 9.7% and 2.5% for small ruminant and large ruminant, respectively, which was considerably significant (*P value*= 0.01).

Conclusions: Due to the higher contamination of milk and dairy product taken from cattle, sheep, and goats it seems that control and prevention programs in sheep and goats must be taken more seriously. Also, since livestock are more kept in rural areas, must lay emphasis on preventive programs including training and hands-on workshops in such areas.

Background

Brucellosis is an important zoonosis with global distribution which can happen in 3 stages: acute, subacute or chronic [1, 2]. The causes of brucellosis is a small, gram-negative, non-motile and facultative intracellular coccobacillus, which can infect a wide range of mammals include cow, sheep, goat, pigs, rodents, marine mammals and human. Brucellosis can affect the reproductive system and lessen the fertility or even cause spontaneous abortion or infertility, especially in cattle. [1, 3]. The humans are infected by Brucella spp. via the gastrointestinal tract, respiratory system, and non-intact skin. The Bacteria disseminate in the body through the blood circulation and lymphatic system [4]. Brucellosis usually presents by fever, sweating, weakness, musculoskeletal pain, lethargy, and weight loss. Also, it can cause local infection in the liver, spleen, bones, and some other organs [5]. This disease has been known for prolonged and long-term illnesses of thousands of faces and naturally identifies ways of transmitting disease and its control can make an important contribution to the health of the community [6]. Six species of Brucella have been identified as disease-causing agents, which B. abortus, B. canis, B. melitensis, and B. suis. capable of causing disease in humans [2]. The human usually infected by consumption of contaminated milk and dairy products [7]. Brucellosis may transmit through non-intact skin, even the placenta, and also it is an airborne disease. 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 [8, 9].

Brucellosis is a zoonosis disease which 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 and Japan and 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 [8, 10]. The countries like Iran, Saudi Arabia, Syria, Jordan, and Oman have the highest incidence of human Brucellosis [11], and the incidence of brucellosis in the Middle East is between 1 to 78 people per 100,000 population [5, 12]. 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 the 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 [13]. Otlu and colleagues 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 exchange of livestock between them [14]. In a study by Forghani et al. In Yazd province – Iran, during the years 2006–2009, 63% of cases had a history of contact with the livestock. After the contact, the most common route of transmission was through the contaminated food and the highest rate was related to housewives (30.8%) [15]. The disease is widespread in all parts of Iran, but its prevalence is not the same in different regions, so that the least incidence reported in the southern regions of Iran and the highest infection rates (98 to 130 cases per 100,000 people) exists in the provinces of Hamadan, Central, East Azarbayjan and Zanjan. This study was designed and conducted to investigate the brucella infection of milk and dairy products have been used in different areas of Hamadan, west of Iran, in local dispensary in unpasteurized form. Ultimately, the purpose of this research is to assist health planners and administrators interconnection to precise and effective control of the disease, taking into account the main routes of transmission, as well plan targeted and hands-on training for rural and also healthcare personnel of rural and countrysides.

Results

out of 291 samples, 12 (4.1%) samples showed brucella growth and after identification and confirmation tests, 9 (75%) samples found to be Brucella melitensis and the remaining 3(25%) were Brucella abortus (Tables 1–3). The Brucella contamination rates in urban, rural and industrial farms found to be 2(1.9%), 10(6.2%) and 0.0%, respectivelly (Table 1). Fischer exact tests did not show any significant defirences in urban and rural areas (P value = 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 other hands, out of 162 samples collected from rural areas, 10 samples showed bacterial growth which found to be 8 cases of B. melitensis and 2 cases of B. abortus. The contamination rates in milk samples was 4%, cheese 2.3% and in cream 9.5%. Fisher's exact test did not show any significant difference in three samples (P value = 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)
Industrial	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)
Fat	2(9.5)	19(90.5)	21(100)
Total: No(%)	12(4.1)	279(95.9)	291(100)

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)

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

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. [16]. Brucellosis, in addition to being a zoonosis disease between humans and livestock, is also transmitted through milk and dairy products. In the present study, a total of 12 samples (4.1%) of dairy products, including milk, cheese, and cream infected by Brucella spp. In a study by Bateni and et al. in Zanjan, out of 299 milk and cheese samples examined by culture method, 5 samples (1.67%) were infected by Brucella [17]. In other reserch reported by Movasagh et al. in Parsabad Moghan region of Ardabil province, Iran, the rate of contamination of raw cattle milk was 37.5% [18]. 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 [19]. Izadi et al. investigated the rate of brucella infection in milk and diry products using Nested PCR technique in Tehran province, Iran. They reported in 34 pasteurized milk samples 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 [20]. It seems that the differences between the results of the current study and previous ones are related to the methodology and the area of researchs.

In Turkey, a study on 1028 brucellosis patients, shown 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 consumption diverse from 62.6–94.6%. 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 accompanying with camel milk drinking. The eating of 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 [21]. 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 [22]. Movasagh et al. taken 50 random samples of cow’s milk and by using ELISA method (Milk ring test) reported 10% of samples have been positive for B. abortus [23]. 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 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 [24]. 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%), while real-time PCR (RT-PCR) technique amplified Brucella-specific DNA from 17 milk samples (7.9%), which 16 of the RT-PCR-positive samples as containing B. melitensis DNA; 1 RT-PCR-positive sample was identified as containing B. abortus DNA [25]. 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 found to be 0% [26]. By considering the high level of contamination in dairy products, it seems that the use of boiled milk to prepare cheese might be an effective way to combat the disease [27].

Conclusions

Our study provided evidence that education with respect to the nature of the brucellosis and the manners of transmission through milk and dairy products is required for farmers and consumers. In addition, the old belief about the usefulness of raw milk than pasteurized milk should be taken into account in light of current scientific information. Consumption of raw milk and unpasteurized dairy products is considered a serious public health hazard. 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 spread of the disease.

Methods

Sampling

This cross-sectional and descriptive-analytical study was performed on milk (227 samples), cheese (43 samples) and creams (21 samples) from traditional or non-pasteurized dairy supply centers in Hamadan [urban and rural areas], and dairy farms. Milk samples collected from local dairy distributors direct from villages and of course unpasteurized and also samples collected from dairy farms which passed all hygienic processes. Regarding cheese samples, half of them collected from dairy product dispensaries with sources in villages and countrysides and another half regular pasteurized cheese. The cream samples were taken from stores or rural houses that were prepared in a traditional and non-pasteurized manner.

Diagnosis and identification of Brucella spp.

To detect Brucella spp. in samples, 10 ml of milk, 100 grams of fresh cheese and cream was taken into sterilized tubes, centrifuged at 3000 rpm for 20 minutes, discard the supernatant, then add 10 ml of Brucella broth medium to the pellet and incubated at 35° C for 24 hours. After the preliminary incubation by a sterile loop, inoculate the sample on medium supplemented with different antibiotics. The following concentrations of antibiotics were added per litre of media: cyclohexaimide (100 mg), bacitracin (25000 units), polymyxin B sulphate (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 at 35 °C for at least seven days; suspected colonies were identified by gram staining and biochemical tests [16, 28].

Data analysis

The obtained data and values are entered to SPSS software (v. 20) and the results were analyzed using descriptive statistics, by performing the Fischer exact test.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Hamadan University of Medical Sciences (No: IRUMSHA. REC.1393.930222646). The written consent taken from all the participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interests

Funding

The Vice-chancellor of Research and Technology, Hamadan University of Medical Sciences, Hamadan, Iran supported financially the study (Grant Number: 930222646). The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

MYA and MMM designed the study and drafted the work. PK contributed to the sample collections and the experimental studies. MYA, MMM and AK analyzed the data. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to acknowledge Vice-chancellor of Research and Technology, and Brucellosis Research Center of Hamadan University of Medical Sciences, Hamadan, Iran, and microbiology laboratory staffs.

Abbreviations

CO2
Carbon dioxide; PCR:Polymerase chain reaction; iELISA:indirect enzyme-linked immunosorbent assay; RT-PCR:real-time PCR

References

1. Cutler, S., Whatmore, A., Commander, N.: Brucellosis. new aspects of an old disease. Journal of applied microbiology 98(6), 1270-81 (2005). 2. Hatami, H.: Brucellosis epidemiology. 2nd National Iranian

Congress ON Brucellosis; 2007 3. Zanganeh, N., Siahpoushi, E., Kheiripour, N., Kazemi, S., Goodarzi, M.T., Alikhani, M.Y. Brucellosis causes alteration in trace elements and oxidative stress factors. Biological trace element research 182(2), 204-8 (2018). 4. Lapaque, N., Moriyon, I., Moreno, E., Gorvel, J.P. Brucella lipopolysaccharide acts as a virulence factor. Current opinion in microbiology 8(1), 60-6 (2005). 5.Tabatabaei, S., Zahraei, M., Ahmadnia, H., Ghotbi, M., Rahimi, F. Principles of disease prevention and Surveillance. Rohe Ghalam Publication Iran 500,173-7 (2006) (Persian). 6. Shoraka, H.E., Hosni, S.H. Epidemiological Survey of brucellosis in the two cities of North Khorasan (Mane and Solmaqan) in 2009. Journal of North Khorasan 2(2,3), 65-72 (2010). 7. Al-Eissa, Y.A. Brucellosis in Saudi Arabia: past, present, and future. Annals of Saudi medicine 19(5), 403-5 (1999). 8.Zoghi, A. Theoretical Overview on human brucellosis. 2nd National Iranian Congress ON Brucellosis-Shahid BeheshtiUniversity of Medical Sciences, 2007 [Persian] 9. Naseri, Z., Alikhani, M.Y., Hashemi, S.H., Kamarehei, F., Arabestani, M.R. Prevalence of the most common virulence-associated genes among Brucella melitensis isolates from human blood cultures in Hamadan Province, West of Iran. Iranian journal of medical sciences 41(5), 422 (2016). 10. Hashemi, S.H., Asadi, F.T., Alikhani, M.Y., Moghimbeigi, A., Naseri, Z. Comparison of serology, culture and polymerase chain reaction (PCR) for diagnosis of human brucellosis. International Journal of Infectious Diseases 45, 476 (2016). 11. Refai, M. Incidence and control of brucellosis in the Near East region. Veterinary microbiology 90(1-4), 81-110 (2002). 12. shirzadi, Z. M., Shirzadi, M. Effective Factors in the control and prevention of brucellosis in the past two decades. 2nd National Iranian Congress ON Brucellosis- Shahid Beheshti University of Medical Sciences 106-108 (2007). 13. Pappas, G., Papadimitriou, P., Akritidis, N. The new global map of human brucellosis. Lancet Infect Dis 6, 91-99 (2006). 14. Otlu, S., Sahin, M., Atabay, H., Unver., A. Serological investigations of Brucellosis in cattle, farmers and Veterinarians in the Kars district of Turkey. Acta Veterinaria Brno 77(1), 117-21 (2008). 15. Forghani, H., NezamHoseyni, S.J.M. Epidemiological Survey of brucellosis in Yazd province since 2003 until 2006. 2nd National Iranian Congress ON Brucellosis-Shahid Beheshti University of Medical Sciences; 19-21 may, 06-108 (2007). 16. Asadi, F.T., Hashemi, S.H., Alikhani, M.Y., Moghimbeigi, A., Naseri, Z. Clinical and diagnostic aspects of brucellosis and antimicrobial susceptibility of Brucella isolates in Hamedan, Iran. Japanese journal of infectious diseases 70(3), 235-8 (2017). 17. Bateni, J., Samadzadeh, R. A survery on the contamination of traditional cheese and milk survey in zanjan city with brucella and E.coli. The Scientific Journal of Zanjan University of Medical Sciences 9(35), 58-65 (2002). 18. Movassagh, M.H., Panahi Azar, D. Contamination rate of cow's raw milk with Brucella abortus in Parsabad region by Milk Ring Test. Journal of Food Hygiene 4(1), 71-6 (2012). 19. Akbarmehr, J. Survey on the contamination of unripened traditional cheese with Brucella in Sarb, Iran. Journal of Faculty of Veterinary Medicine, University of Tehran 58(3), 203-206 (2003). 20. Izadi, A., Moslemi, E., Tabatabaei Panah, A. S., Kheiri Manjili, H. Brucella spp. Detection in dairy products using Nested and Hemi Nested PCR techniques. Annals of Biological Research, 5 (1), 24-131(2014). 21. Dadara, M., Shahalia, Y., Whatmoreb, A.M. Human brucellosis caused by raw dairy products: A review on the occurrence, major risk factors and prevention. International Journal of Food Microbiology 292, 39–47 (2019). 22. Khalili, M., Aflatoonian, M.R., Salari Aliabadi, F., Abshenas, J. Drucilla contamination in raw milk by polymerase chain reaction. Tehran Univ Med J [TUMJ] 74(7), 517-21(2016). 23. Movassagh, H. A Survey of brucella abortus contamination of cow milk in ilkhchi region with Elisa method. Food Technology & Nutrition 10 (1), 97-101(2012). 24. Silva,

J., de Moraes, C.M., Silva, C.L., Sales, G.A., Kid, L.B., Matos, P.C. Brucella abortus detected in cheese from the Amazon region: Differentiation of a vaccine strain [B19] from the field strain in the states of Para, Amapa and Rondonia, Brazil. Pesq.Vet. Bras 36(8), 705-710 (2016). 25. Wareth, G., Melzer, F., Elschner, M.C., Neubauer, H., Roesler, U. Detection of Brucella melitensis in bovine milk and milk products from apparently healthy animals in Egypt by real-time PCR.J Infect Dev Ctries 8(10), 1339-43(2014). 26. Alper, S., Nesrin, C. All Rights Reserved Bacterial contamination in fresh white cheeses sold in bazaars Canakkale, Turkey. International Food Research Journal 20(3), 1469-72 (2013). 27. Celebi, O., Celebi, D., Balkan, C.E. Effects of Boiling Dairy Products on Human Brucellosis. Eurasian J Med 45(2), 73-76 (2013). 28. Bahmani, N., Mirnejad, R., Arabestani, M.R., Mohajerie, P., Hashemi, S.H., Karami, M. Comparison of PCR-RFLP and PFGE for determining the clonality of Brucella isolates from human and livestock specimens. Saudi Journal of Biological Sciences 26, 256–262 (2019).