



Investigating the Prevalence of Brucellosis, as an Occupational Disease, in Employees of Traditional Dairy Workshops in Sarab

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ABSTRACT

Aims: Brucellosis is one of the most common diseases between humans and animals (zoonosis); one of its transmission routs is through the consumption of infected cattle dairy products. Since Sarab city has a large cattle population, and traditional dairy production and processing practices are still prevalent in this region and are known as tourist attractions and souvenirs of the region, this study aimed to investigate the prevalence of infection in employees of traditional dairy products workshops in Sarab in 2018.

Materials & Methods: This descriptive cross-sectional study was conducted on 196 employees of traditional dairy production and packaging workshops. Blood samples were obtained from all subjects in order to determine the serological status of the patients. After sera isolation, Rose Bengal, seroagglutination in tube (Wright), 2-mercaptoethanol (2ME), and ELISA tests were used to evaluate them.

Findings: In this study, the disease prevalence in the subjects with the mean age of 33±4.2 years was determined as 2, 1, 1, and 1% using Rose Bengal, Wright, 2-mercaptoethanol, and ELISA tests, respectively. The mean knowledge score of the employees in dairy products workshops was 31±5.7, and by increasing age and duration of work, knowledge about brucellosis was also increased

Conclusion: In this study, the mean knowledge score showed the average knowledge of the subjects about brucellosis. The disease prevalence in this occupational group was lower than that reported in other studies conducted on other at-risk occupational groups in other parts of Iran and the world. Therefore, health personnel should take the necessary measures against brucellosis.

Keywords: Brucellosis, Occupational disease, Prevalence, Dairy production workshop, Awareness

CITATION LINKS

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Introduction

Brucellosis is one of the most common diseases between humans and animals (zoonosis), caused by *Brucella* bacteria. The disease is also known as Mediterranean fever ⁽¹⁾.

The main transmission route of the disease to humans is through the consumption of unpasteurized milk and dairy products such as cheese, ice cream, and raw milk, produced from infected animals. This disease could also be transmitted to humans in contact with the tissues, blood, urine, and secretions of infected animals through unhealthy hands not protected by gloves; inhalation of bacteria aerosolized in the air of pen, slaughterhouse, and laboratory; accidental inoculation of animal vaccine; placenta; transfusion of blood and bone marrow; and even through the conjunctiva of the eye ⁽²⁻⁴⁾. The incubation period of brucellosis infection is usually 1 to 2 weeks, but in some patients, it may take several months ⁽⁵⁾. Brucellosis in humans could cause localized purulent infection in the liver, spleen, bones, and some other organs, it is known as a disease of a thousand faces as a result of various long-term complications resulting from various organs involvement. Extensive clinical manifestations have made the disease diagnosis difficult ^(6, 7).

Brucellosis is a health and economic problem that is often unknown in many parts of the world, including the Mediterranean, Middle East, and Persian Gulf countries. Although the disease prevalence has decreased in some societies in recent years, it is still a serious threat to human health, especially in developing countries, including Iran ^(8, 9).

According to the World Health Organization, 500,000 patients with brucellosis are annually identified and reported ⁽¹⁰⁾. The disease is endemic in Iran, and in terms of the brucellosis prevalence, Iran is ranked fourth in the world and first among the

eastern Mediterranean countries ^(11, 12). The cumulative incidence of infection in human population of the country is annually about 34 cases per 100,000 people, and due to the multiple transmission routes of the disease to humans and the impossibility of using pasteurized dairy products for the whole population, the disease has heterogeneously become prevalent within the human society, especially in the rural areas ⁽¹³⁾. Accordingly, brucellosis could be considered as an occupational disease; people at risk of brucellosis are veterinarians, ranchers, slaughterhouse staff, and those exposed to raw dairy products ⁽¹⁴⁾.

The diagnosis of brucellosis could not be based solely on clinical symptoms because it is confused with other diseases such as malaria, typhoid, and leptospirosis. Therefore, the definitive diagnosis of this disease is done by culturing the microorganisms causing the disease or by using serological and molecular methods ^(15, 16).

The common method for the diagnosis of this disease is agglutination (Wright, Coombs' Wright, and 2-mercaptoethanol) by standard tube agglutination (STA). The anti-*Brucella* ELISA (IgM, IgG) method could also be used because of its speed and availability and maintenance of its kits for a long time ⁽¹⁷⁾. The previous studies results showed that ELISA (IgM, IgG) test was more sensitive than agglutination test; however, its specificity was not low. Therefore, ELISA (IgM, IgG) test could be used as a diagnostic test for brucellosis ⁽¹⁸⁾.

Sarab city with a population of more than one hundred thousand people is located on the east of East Azarbaijan province and is the third largest city in the province. The city is considered as an important animal husbandry hub in the province because of its favorable climate and rich and lush rangelands. The production level of dairy

products in this city is very high both in quantity and quality and has given Sarab a privileged position. Unfortunately, despite all the above features, in this city, like in many other parts of the country, the prevalence of brucellosis is very high⁽¹⁹⁾.

Objectives: Given the large livestock population in Sarab and considering the fact that livelihood of a major part of the rural population in this area is through the production of dairy products from their livestock using traditional dairy production methods, investigating the prevalence of brucellosis in this occupational group of the city is of great importance. Identifying the disease status in the area could be helpful in planning to control and reduce the disease. Since there was no published study on the prevalence rate of brucellosis in the human population of Sarab, the number of chronic and acute cases and also the level of *Brucella* antibody in at-risk groups were determined to take more effective steps in order to prevent and reduce the disease prevalence. Therefore, by providing accurate information about the current status of this disease, more effective interventional programs could be implemented to address the disease and improve the health and sanitation of the human population in this city.

Materials and Methods

This descriptive cross-sectional study was carried out on 196 employees of traditional dairy production and packaging workshops in Sarab as well as those who were related to traditional dairy products due to their occupation. Demographic characteristics and information related to the subjects, including age, sex, place of residence, work experience, history of unpasteurized dairy use, use of protective equipment during work, keeping livestock at home, contact with livestock, history of infection with brucellosis in person or family as well as the

level of awareness about brucellosis were collected and recorded by a questionnaire. Data related to the subjects' awareness were collected using a researcher-made questionnaire, the validity and reliability of which were confirmed. Given the low education level of the people in the region, a 3-point Likert type rating scale was used, and the answers to the questions were placed in three levels ("yes", "no", and "I don't know"). In the awareness (20 questions) section, the correct, wrong, and "I don't know" answers were given 2, zero, and 1 scores, respectively, and the total score for each person ranged from zero to 40. To determine the serological status of the patients, after receiving informed consent of each person, 5 mL of venous blood was collected by a syringe and discharged into a sterile clot tube and then transferred to central laboratory of Sarab University of Medical Sciences. Then the blood serum was separated by centrifugation and stored in a 1.5 mL microtube at 20 °C. Rose Bengal, sero-agglutination in tube (Wright), 2-mercaptoethanol (2ME), and ELISA (IgG and IgM) tests were performed on the serums. As a first step, Rose Bengal test was performed on the serum samples, and positive and suspicious samples were evaluated by supplementary tests of Wright and 2ME. Serum grade of 1.80 and higher was considered as positive in Wright test, and in 2ME test, titer equal to or greater than 1.40 was considered as active disease, and titer less than 1.40 was considered as inactive disease^(20, 21). Finally, serum samples were analyzed by ELISA test to determine the class of IgG and IgM anti-*Brucella* antibodies as well as the disease activity and inactivity. All data were analyzed using SPSS software Ver. 21 and descriptive statistics and chi-square test.

Findings

This study was conducted on 196 employees

of traditional dairy products workshops in Sarab region, including 184 (93.8%) men and 12 (6.2%) women with the age ranges from 19-62 years and the average age of 33 ± 4.2 years. Of whom, 88 (44.8%) were single, and 108 (55.2%) were married. In terms of educational level, 9 (4.8%) cases were illiterate, 140 (71.4%) cases did not have high school diploma, 39 (19.8%) had high school diploma, and 8 (4%) were in the grades higher than high school diploma. Of whom, 59 (30%) were urban, and 137 (70%) were rural. The average work experience of the study population was 6.23 ± 2.44 years, 62 (44.8%) cases had a history of direct contact with livestock in the past year, and 41 (21%) were keeping livestock including sheep, goats, and cows in their residence place. Also, 3 (1.5%) cases had a history of brucellosis infection in their families (Table 1).

Among the study population, 67 (34.1%) had a history of training on brucellosis and had received the most up-to-date recommendations from health care personnel about this disease. The mean knowledge score of the employees in dairy products workshops was 31 ± 5.7 , and the level of awareness was significantly related to higher education ($P < 0.05$). This study showed that by increasing age and duration of work, the level of awareness about brucellosis also increased. The workers' residence place had no significant relationship with their awareness level about brucellosis.

Out of 196 serum samples tested, Rose Bengal was positive for 4 (2%) samples, and 2ME and Wright tests were positive for 2 samples. The ELISA test results almost confirmed the 2ME and Wright tests results and showed that 2 (1%) samples were seropositive. Out of 2 seropositive individuals, one had active form (IgM), and the other one had inactive form (IgG) of the disease (Table 2).

Discussion

Many studies have been conducted on the prevalence of brucellosis in at-risk occupational groups worldwide; the large number of these studies, in addition to demonstrating the importance of this disease, shows that brucellosis is a widespread disease, and its outbreak is remarkable in some areas of the world, such as the Middle East; thus, it is necessary to have right policies and data on subjects under study as well as on the epidemiology of the disease in order to be able to control, prevent, or eliminate the disease, like any other disease.

In this study, the mean awareness score indicated the subjects' average knowledge about brucellosis. The results showed that by training, the subjects' awareness about brucellosis also increased, consistent with the results of Shahnvari et al. ⁽²²⁾.

In this seroepidemiological study, the disease prevalence in the employees of traditional dairy products workshops in Sarab was determined as 2, 1, 1, and 1% using Rose Bengal, Wright, 2-mercaptoethanol, and ELISA tests, respectively. In this study, the disease prevalence rate was lower than that reported in other studies conducted on other at-risk occupational groups in other geographical areas of Iran and the world; for example, in Kerman among people working in ranches in 2012, in Gilan among slaughterhouse employees in 2008, in Turkey among ranchers in contact with cows during 2004-2006, and in Pakistan among ranchers and slaughterhouse workers and rural people in 2007, seroprevalence rate was reported as 3.2, 9.8, 17.88, and 11%, respectively ^(4, 23-25).

In addition to the difference in geographical location, type of test used, level of awareness, vaccination, and type of killing infected animals, the difference in the prevalence rate in this study compared to other studies

Table 1) Relationship between awareness and demographic and occupational characteristics of the employees with seropositive brucellosis in dairy products workshops

Variable		Number	Percentage	Number of Positive Serology	Percentage of Positive Serology	P value
Gender	Male	184	93.8	2	1	.124
	Female	12	6.2	0	0	
Age	Under 25 yrs old	48	24.4	0	0	.346
	26-35 yrs old	72	36.7	1	1.3	
	36-45 yrs old	39	19.8	1	2.5	
	Over 45 yrs old	37	18.8	0	0	
Marital status	Single	88	44.8	1	1.1	.041
	Married	108	55.2	1	0.9	
Place of residence	Urban	59	30	0	0	.112
	Rural	137	70	2	1.4	
Education	Illiterate	9	4.8	0	0	.237
	Under diploma	140	71.4	2	1.4	
	Diploma	39	19.8	0	0	
	University	8	4	0	0	
Work experience	Under 5 yrs	78	39.7	1	1.2	.053
	5-10 yrs	84	42.8	1	1.1	
	10-20 yrs	22	11.2	0	0	
	Above 20 yrs	12	6.3	0	0	
History of raw milk consumption	Yes	28	14.3	1	3.5	.345
	No	168	85.7	1	0.6	
Contact with livestock in the past year	Yes	62	44.8	1	1.6	.092
	No	134	55.2	1	0.7	
Keeping livestock in house	Yes	41	21	1	2.4	.164
	No	155	79	1	0.6	
History of infection with brucellosis in family	Yes	3	1.5	0	0	.037
	No	193	98.5	2	1	
History of training	Yes	67	34.1	0	0	.047
	No	129	65.9	2	1.5	
Level of awareness	Poor	82	41.8	2	2.4	.021
	Medium	71	36.2	0	0	
	Good	43	22	0	0	

Table 2) Absolute and relative frequency distribution of IgM and IgG antibodies based on 2-mercaptoethanol and ELISA tests results

Frequency of Positive Results Type of Antibody	Percentage of Total Samples	2ME test Number	Percentage	ELISA test Number	Percentage
IgG	0.5	1	0.5	1	0.5
IgM	0.5	1	0.5	1	0.5
Total	1	2	1	2	1

could be due to including only at-risk occupational groups as in Iran, butchers and slaughterhouse staff are more at risk^(1, 26). All serologically positive individuals had poor awareness of brucellosis, which may be due to the fact that people with poor awareness of brucellosis do not take the necessary preventive measures and are more likely to be infected with the disease. By wearing safe clothing, latex or plastic gloves, and mouth and nose masks; training employees; pasteurizing milk before starting processing; and industrializing dairy production workshops, it is possible to reduce the prevalence rate of infection among these hardworking people.

In this study, all positive individuals were male, which may be due to the large number of male staff employed in dairy products workshops. The higher disease prevalence in men was consistent with the studies by Mehdi Mohammadian et al.⁽²⁷⁾, Almasi Hashiani et al.⁽²⁸⁾, and Elbeltagy in Saudi Arabia⁽²⁹⁾. Although it could be said that brucellosis is an occupational disease that is more common in men, it is worth noting that in some areas, women work alongside men in jobs such as producing dairy products, and even if brucellosis is considered as an occupational disease, it should be noted that it is not necessarily specific to men and could also infect women.

In this study, the average age of the patients was 34 years old. Given the economic and social activities of this age group, the

importance of combating this disease in the age groups of 20 and 30 years is more pronounced. All patients were also from rural areas, which is consistent with a number of other studies results^(2, 30).

According to the World Health Organization, livestock vaccination is the only appropriate method and the first step in controlling brucellosis⁽³¹⁾. Prevention of human brucellosis could also be done in two ways: not contacting with infected livestock and not consuming infected dairy products⁽³²⁾. According to a global standard, the prevalence of brucellosis in humans in any country is highly correlated with the prevalence of the disease in animals of that country⁽³³⁾, indicating the importance of vaccination and treatment programs (in pets and companion animals) under the supervision of veterinary networks throughout the country, especially in areas with high infection prevalence.

Conclusion

In summary, it could be stated that if these people have been infected solely because of their occupation and contact with infected dairy products, the number of livestock infected with brucellosis in the Sarab area may be lower; also, health and education measures and disease control might have been taken well. Therefore, in order to eradicate the disease in livestock, which eventually leads to the eradicate of the disease in humans, the health care personnel should take the necessary measures to

raise the awareness of employees in the workshops, to raise the level of occupational health against brucellosis, and to complete the livestock vaccination coverage in the area with the cooperation of the Veterinary Office.

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References

1. Azizi F, Hatami H, Janghorbani M. Epidemiology and control of common diseases in Iran. Tehran: Eshtiagh Publications. 2000:602-16.
2. Farahani S, SHAHMOHAMADI S, Navidi I, Sofian S. An investigation of the epidemiology of brucellosis in Arak City, Iran),2001-2010 .(2012.
3. Heymann D. Control of communicable diseases manual: an official report of the American Public Health Association. Washington DC. World Health Organization/American Public Health Association. 2004;2.
4. Mohammadkhani M, Sharifi H, Rashidi H, Nabipour A, Jahanshahi M. Seroepidemiology of brucellosis in industrial and semi-industrial dairy personnel and veterinary network staff in kerman, 2012. Iranian Journal of Epidemiology. 2015;10(4):54-61.
5. Young EJ. An overview of human brucellosis. Clin Infect Dis. 1995;21(2):283-9; quiz 90.
6. Talwani R, Gilliam BL, Howell C. Infectious diseases and the liver. Clinics in liver disease. 2011;15(1):111-30.
7. Cesur S, Çiftçi A, Sözen T, Tekeli E. A case of epididymo-orchitis and paravertebral abscess due to brucellosis. Journal of Infection. 2003;46(4):251-3.
8. Hadadi A, Rasoulinejad M, Haji Abdolbaghi M, Mohraz M, Khashayar P. Clinical profile and management of brucellosis in Tehran-Iran. Acta Clinica Belgica. 2009;64(1):11-5.
9. Hashemifar I, Yadegar A, Jazi FM, Amirmozafari N. Molecular prevalence of putative virulence-associated genes in *Brucella melitensis* and *Brucella abortus* isolates from human and livestock specimens in Iran. Microbial pathogenesis. 2017;105:334-9.
10. Hull NC, Schumaker BA. Comparisons of brucellosis between human and veterinary medicine. Infection ecology & epidemiology. 2018;8(1):1500846.
11. Ramin B, MacPherson P. Human brucellosis. Bmj. 2010;341:c4545.
12. Roya N, Abbas B. Colorectal cancer trends in Kerman province, the largest province in Iran, with forecasting until 2016. Asian Pacific Journal of Cancer Prevention. 2013;14(2):791-3.
13. Kaboutari J, Sharifi H, Yousefzade A, Mashayekhi K, Khoshkam M, Afsharipour

- N. Seroprevalence of ovine and caprine anti-Brucella antibodies in south of Kerman province (Iran, 2012). *Journal of Veterinary Research*. 2015;70(4).
14. Çetinkaya F, Naçar M, Aydın T, Koç N, Gökahmetoğlu S. Prevalence of brucellosis in the rural area of Kayseri, Central Anatolia, Turkey. *International Journal of Infectious Diseases*. 2006;10(2):179-81.
 15. Richtzenhain LJ, Cortez A, Heinemann MB, Soares RM, Sakamoto SM, Vasconcelos SA, et al. A multiplex PCR for the detection of *Brucella* spp. and *Leptospira* spp. DNA from aborted bovine fetuses. *Veterinary Microbiology*. 2002;87(2):139-47.
 16. Leal-Klevezas DS, Martínez-Vázquez IO, Lopez-Merino A, Martínez-Soriano JP. Single-step PCR for detection of *Brucella* spp. from blood and milk of infected animals. *Journal of clinical microbiology*. 1995;33(12):3087-90.
 17. Mohraz M, Kariminia A, Sarafnejad A. The Evaluation of Serologic Test (Elisa) in Brucellosis identification at Imam Khomani Hospital. *Infectious and Tropical disease*. 2003;8(23):10-3.
 18. Mathai E, Singhal A, Verghese S, D'Lima D, Mathai D, Ganesh A, et al. Evaluation of an ELISA for the diagnosis of brucellosis. *Indian J Med Res*. 1996;103:323-4.
 19. Akbarmehr J, Ghiyamirad M. Serological survey of brucellosis in livestock animals in Sarab City (East Azarbayjan province), Iran. *African Journal of Microbiology Research*. 2011;5(10):1220-3.
 20. Chegeni AS, Ezatpour B, Saki M, Mokhayeri H, Adavi S, Nasiri E, et al. Seroepidemiology of human brucellosis in nomads in a rural area of Iran. *Asian Pacific Journal of Tropical Disease*. 2014;4(4):333-6.
 21. Hashemi SH, Torkaman Asadi F, Alikhani MY, Naseri Z. Comparison of culture and serological methods for the diagnosis of brucellosis. *Avicenna Journal of Clinical Medicine*. 2015;22(1):37-42.
 22. Shahnavaizi M, Gholamreza M, Ansari-Moghadam A, Raeisy D, Varnamkhasti Khashei F. Preventive Behaviors of Brucellosis in Khash City Ranchers Based on Health Belief Model. *Iranian Journal of Health Education and Health Promotion*. 2017;4(4):281-8.
 23. Nikokar I, Hosseinpour M, Asmar M. Seroprevalence of Brucellosis among high risk individuals in Guilan, Iran. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*. 2011;16(10):1366.
 24. 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*. 2008;77(1):117-21.
 25. Hussain I, Arshad MI, Mahmood MS, Akhtar M. Seroprevalence of brucellosis in human, cattle, and buffalo populations in Pakistan. *Turkish Journal of Veterinary and Animal Sciences*. 2008;32(4):315-8.
 26. Vahdat K, Jafary SM, Hashemi SM. Seroepidemiological prevalence of brucellosis in livestock breeders of the central rural area of Bushehr province 2003-4. *ISMJ*. 2006;9(1):51-8.
 27. Mohammadian M, Salehiniya H, Kazaei S, Ramazanpour J, Mohammadian-Hafshejani A. Epidemiological characteristics and incidence rate of brucellosis in Isfahan province, Iran, 2012. *Journal of Isfahan Medical School*. 2015;33(355):75-82.
 28. Almasi-Hashiani A, Khodayari M, Eshtrati B, Shamsi M. Factors affecting the interval between the onset and diagnosis of brucellosis in Markazi Province, Iran (2010-11). *Journal of Arak University of Medical Sciences*. 2012;14(7):21-30.
 29. Elbeltagy K. An epidemiological profile of brucellosis in Tabuk Province, Saudi Arabia. 2001.

30. Ghasemi B, Mohammadian B, Majidpour M. Epidemiology of human and animal brucellosis in Kurdistan Province in 1997-2001. 2004.
31. Joint FAO/WHO Expert Committee on Brucellosis [meeting held in Geneva from 12 to 19 November 1985]: sixth report. 1986.
32. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, et al. International variation in prostate cancer incidence and mortality rates. *European urology*. 2012;61(6):1079-92.
33. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: a re-emerging zoonosis. *Veterinary microbiology*. 2010;140(3-4):392-8.