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Seroprevalence and Risk Factors of Brucellosis in Abattoir Workers in Fars Province, Iran

Alireza Zakeri, Maryam Montaseri, Seyed Shahram Shekarforoush

Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

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Abstract

Background & Objectives: Brucellosis remains an important occupational zoonotic disease, especially in developing countries. The disease is endemic in Iran and the Fars province. One of the main routes of brucellosis infection is at slaughterhouses, where the workers directly contact infected animals. This study was designed to estimate the seroprevalence of brucellosis among slaughterhouse workers in the Fars province, Iran.

Materials & Methods: Ninety blood samples were collected from workers of two livestock slaughterhouses (Marvdasht and Kazeroon), in Fars, Iran. The sera were assessed for the Rose Bengal test (RBT), as a screening test for brucellosis, and the positive samples were subjected to the Wright test. The positive Wright samples were finally tested for the 2-mercaptoethanol (2-ME) agglutination test.

Results: Brucellosis prevalence was 13.33% using RBT and 4.44% of the workers showed active brucellosis. No significant relationship was found between the questionnaire variables and brucellosis tests; exceptionally, there was a relationship between the workers' statements regarding having had brucellosis and RBT (P=0.01).

Conclusion: Our study highlights the practical application of serological tests, including RBT, Wright, and 2-ME as a simple strategy to monitor brucellosis and to diagnose and treat its active form in endemic regions. Although a small frequency of the disease was found, it could cause significant health and economic damage to humans and animals in endemic areas. Furthermore, taking enough protective measures is highly recommended for slaughterhouse workers to prevent human brucellosis.

Keywords: Brucellosis, Seroprevalence, Abattoirs, Iran

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Introduction

Brucellosis, caused by the genus *Brucella*, is currently one of the most common zoonotic infections worldwide, frequently occurring in countries where regular and effective eradication programs are not present (1). The high-risk areas include North and East Africa, Eastern Europe, the Mediterranean region, south and Central Asia, and the Middle East, such as Iran (2). Most parts of Iran are endemic for human brucellosis with a pooled incidence of 0.001%, annually (3). The disease is categorized into four types: very high, high, moderate, and low in provinces of Iran. Accordingly, Fars province is classified into the moderate incidence, 11-20 cases per 100,000 populations (4). *Brucella* infection can primarily occur through inhaling the organisms and d irect

Corresponding Author: Maryam Montaseri, Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
Email: m.pourmontaseri@shirazu.ac.ir

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contact with the placenta, blood, urine, aborted fetus, and vaginal discharges of infected animals, especially goats, cattle, and sheep (1, 5-10). Occupations related to livestock are strongly highlighted for brucellosis, comprising farmers, abattoir workers, butchers, veterinarians, and laboratory workers (9, 11). Working at abattoirs, as a risk factor, has been associated with brucellosis seropositivity in various countries (12-14). Abattoir workers are at most risk of infection via inhalation of infected aerosols, open wounds on bare hands, and splashing of infected fluids (15, 16). In Iran, brucellosis seroprevalence has been reported as 12.3% in Hamadan (17), 17% in Ahvaz (18), and 31.83% in Lorestan (19) among high-risk occupational groups.

Brucellosis may manifest as acute, sub-acute, and chronic in humans according to the duration of the clinical symptoms (20). In chronic brucellosis, symptoms such as myalgia, weakness, fatigue, arthralgia, and endocarditis usually last more than one year (21). As brucellosis can mimic various multisystem diseases, it may be overlooked, misdiagnosed, and not properly treated (22, 23). This problem stands out especially in most low and middle-income countries, without adequate healthcare infrastructure and public awareness. Screening methods of brucellosis in high-risk occupational groups are imperative for early diagnosis and treatment (24). Different methods, including culture, molecular and serological tests, can detect *Brucella* spp. The serological techniques encompass the Rose Bengal test (RBT), Standard Tube Agglutination Test, 2-mercaptoethanol (2-ME) ag-glutination test, and Enzyme-Linked Immunosorbent Assay (ELISA) (5). The culture method is considered the gold standard; however, bacterial growth is difficult and time-consuming. Serological tests are used to screen and confirm brucellosis in clinical samples. They are rapid, safe, and valid tests commonly used to monitor the prevalence of brucellosis in an area. RBT is primarily considered for screening of the infection and confirmed by the subsequent agglutination tests (24, 25).

Some sero-epidemiological investigations of brucellosis ha ve been performed among high-risk occupational groups such as farmers, slaughterhouse workers, butchers, and veterinarians in some areas of Iran (18, 19, 26, 27). However, there is no publication on brucellosis prevalence among slaughterhouse workers in Fars province, Iran, to the best of our knowledge. Therefore, this study intended to evaluate the seroprevalence of brucellosis among slaughter-house workers in Fars province, Iran.

Materials & Methods

Blood sampling

In this cross-sectional study conducted in 2021, the prevalence of brucellosis among slaughterhouse staff was assessed in Fars province, south-central Iran. A total of 90 blood samples were obtained from workers at two livestock slaughterhouses (Marvdasht and Kazeroon). About 5 mL of blood was collected into vacuum tubes without anticoagulant, and promptly transported to the laboratory under refrigerated condition. In the laboratory, after centrifugation (3000 g, 15 min) of the clot blood samples, the sera were separated and stored at -20 °C until use (28, 29).

Rose Bengal Test

The sera samples were applied for serological brucellosis tests. The sera were first screened using an RBT kit (Pasteur, Iran), according to the manufacturer's instructions. Briefly, the sera samples and the reagent were placed at room temperature. An amount of 30 µL of the serum was thoroughly mixed with an equal volume of the antigen on a glass slide and gently shaken for four minutes. Any visible agglutination was considered positive. To validate the accuracy of the study, positive and negative controls were used and the test was conducted in duplicate (28).

Standard tube agglutination test (Wright assay)

The Wright assay was applied to positive samples obtained from the RBT to detect specific antibodies immunoglobulin (Ig)M and IgG. Each positive Wright test was then subjected to a 2-ME agglutination test. The Wright test was implemented

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using a Wright agglutination tube kit (Pasteur, Iran), according to the manufacturer's instructions. Briefly, the sera were prepared after a 2-fold serial dilution with phosphate buffered saline (PBS) (pH=7.4, 1:20 – 1:5120 dilution). After that, 0.5 mL of *B. abortus* antigen was added to each tube, and then incubated at 37°C for 24-48 h. The tubes were finally compared with the positive control. A serum titer ≥ 1:80 was considered positive (19, 30). Positive and negative controls were used and the test was performed in duplicate.

Mercaptoethanol agglutination test

Those tubes with positive Wright tests were assessed for the 2-ME agglutination test to detect IgG antibody titers. The 2-ME test was carried out precisely according to the procedure of the 2-ME test kit (Pasteur, Iran), similar to the procedure for the Wright test. The sera were diluted 1:2, followed by 2-fold serial dilution. Then, 0.5 mL of 2-ME antigen was added to each tube. The tubes were incubated for 24-48 h at 37 °C. A positive control was also included. The titer ≥ 1:40 was considered positive (29). According to the national guideline against brucellosis, the Wright titer ≥ 1:80 and 2-ME titer ≥ 1:40 indicate active brucellosis (31). Positive and negative controls were used and the test was implemented in duplicate.

Questionnaire

The workers filled out a questionnaire at the time of blood sampling. The questionnaire content was designed based on previous studies on slaughterhouse workers (32, 33). The questionnaire included socio-demographic questions (name, age, living place, education level, duration of employment), epidemiological data (history of contracting brucellosis, contact with an aborted fetus, consumption of dairy products), and clinical symptoms (fever, chills, and malaise, cardiac problems). To determine the content validity, the questionnaire was checked and edited by some infectious disease specialists.

Statistical analysis

The data were analyzed using IBM SPSS (Statistical Package for the Social Sciences) version 18. Qualitative statistics were used for frequency percentages. A chi-square analysis was applied to examine the association between variables and serological tests. A $p < 0.05$ was considered statistically significant.

Results

In RBT, 12/90 (13.33%) sera samples were positive. Out of RBT-positive sera, 9/12 (75.00%) were positive (antibody titer ≥ 1:80) for the Wright test. In the 2-ME test, 4/9 (44.44%) of samples were positive (antibody titer ≥ 1:40), out of those positive in the Wright test (Table 1). Generally, 4/90 (4.44%) of the workers showed active brucellosis.

Table 1. The prevalence of brucellosis in slaughterhouse workers according to Rose Bengal, Wright, and 2-ME tests

Test	Positive (%)	Negative (%)	Total (%)
RBT	12 (13.33)	78 (86.67)	90 (100)
Wright	9 (75.00)	3 (25.00)	12 (100)
2-ME	4 (44.44)	5 (55.56)	9 (100)

RBT: Rose Bengal test, 2-ME: 2-mercaptoethanol

According to the questionnaire results, the abattoir workers had a mean age (± standard deviation) of 41.1±1.1, with a range of 20 to 62 years old. The mean (± standard deviation) duration of employment in the slaughterhouses was 11.1±7.2 years, ranging from three months to 45 years. The frequency of the brucellosis tests (RBT and 2-ME) according to the variables is detailed in Table 1. The Chi-squared analysis inferred a significant relationship between the abattoir staff positive history of brucellosis and positive RBT ($p < 0.05$) (Table 2). Moreover, all workers (n=90) wore gowns and boots, but none of

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Table 2. The relationship between brucellosis tests (Rose Bengal and 2-ME) and the demographic characteristics of the slaughterhouse workers

Risk factors	Brucellosis tests					
	Positive RBT			Positive 2-ME		
	No.	No. (%)	*p* value	No.	No. (%)	*p* value
Living place			0.26			0.14
Urban	70	11 (15.7)		11	3 (27.3)	
Rural	18	1 (5.6)		1	1 (100)	
Age			0.22			0.39
20-40	45	4 (8.9)		4	2 (50.0)	
41-62	43	8 (18.6)		8	2 (25.0)	
Education			0.92			1
Lower than high school diploma	63	8 (14.3)		9	3 (33.3)	
High school diploma	22	3 (13.6)		3	1 (33.3)	
Associate degree	3	0 (0.0)				
Work experience (year)			0.28			0.27
1-10	56	10 (15.4)		10	4 (40.0)	
11-20	10	2 (20.0)		2	0 (0.0)	
≥21	13	0 (0.0)		0	0 (0.0)	
Other livestock-related jobs			0.44			0.46
Yes	14	1 (7.1)		1	0 (0.0)	
No	74	11 (14.9)		11	4 (36.4)	
Line of slaughtering			0.55			0.22
Cattle	15	1 (6.7)		1	1 (100)	
Sheep and goat	19	2 (10.5)		2	0 (0.0)	
both	54	9 (16.7)		9	3 (33.3)	
Getting brucellosis			0.01			1
Yes	19	6 (31.6)		6	2 (33.3)	
No	69	6 (8.7)		6	2 (33.3)	
Systemic signs			0.08			0.58
Yes	5	2 (40.0)		2	1 (50.0)	
No	83	10 (12.0)		10	3 (30.0)	
Cardiac/bone problem			0.12			0.68
Yes	21	5 (23.8)		5	2 (40.0)	
No	67	7 (10.4)		7	2 (28.6)	
Contact with aborted fetus			0.15			0.27
Yes	57	10 (17.5)		10	4 (40.0)	
No	31	2 (6.5)		2	0 (0.0)	
Consumption of traditional dairy			0.47			0.39
Yes	66	8 (12.1)		8	2 (25.0)	
No	22	4 (18.2)		4	2 (50.0)	

Obtained from Chi-square test, No: number, RBT: Rose Bengal test, 2-ME: 2-mercaptoethanol

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them (n=90) wore gloves, masks, and goggles. As the positive cases had symptoms (myalgia, weakness, fatigue, arthralgia, and endocarditis) lasting more than one year, they were considered to have chronic disease (21). However, based on the laboratory findings (antibodies titers), the brucellosis was active in the positive cases, requiring treatment.

Discussion

This study surveyed the prevalence of brucellosis, the main occupational-related zoonotic disease, among 90 slaughterhouse personnel in Fars, Iran. The result showed 13.33% seropositivity for RBT, with 4.44% (4/90) of the workers testing positive for active brucellosis. Karimi et al. (34) evaluated *Brucella* antibodies in a high-risk population (20 butchers and 25 slaughterers) in Shiraz in which 10% of abattoir staff were positive for RBT, and 6% showed active brucellosis (2-ME ≥ 1:20). This finding aligns with the results of our study, suggesting that brucellosis remains a prevalent issue in Fars province even after 21 years, not successfully eradicated in this region. Iran is a developing country, located in the Eastern Mediterranean, an endemic region for brucellosis (35). Numerous reasons are implicated in the failure of the eradication programs in Iran, including: 1) insufficient financial support for animal vaccinations, lack of permanent monitoring and slaughtering programs, as well as lack of compensating animal owners; 2) insufficient attention paid to zoonotic diseases by the veterinary organization and other relevant authorities; 3) insufficient cooperation of other organizations and social media with the veterinary organization to promote disease control and preventive goals (31).

There are several reports of brucellosis prevalence among slaughterhouse workers and butchers in some provinces of Iran. These reports comprise high to low seroprevalence, including 43.75% in Lorestan (19), 30.3% in Khorasan (36), 14.4% in Kermanshah (8), 13.1% in Hamadan (17), and 12% in Kurdistan (37). The results of this study were similar to those of Kermanshah, Hamadan, and Kurdistan regions. In a meta-analysis reviewing livestock-related occupational exposure to brucellosis from 2000 to 2022, brucellosis prevalence was found to be 14%, and among different occupational groups, slaughterhouse workers showed the highest prevalence rate of brucellosis (20%) (1). Various serological prevalence rates of brucellosis have been reported among abattoir personnel in different parts of the world; for instance, 75.2% in Egypt (38), 4.4% in Uganda (39), 37.6% in Algeria (40), 21.7% in Pakistan (41), 19.69% in India (42), and 6.1% in South Korea (32). The highest prevalence of brucellosis has been reported in the Middle East region such as Iran, Egypt, Iraq, Saudi Arabia, and Turkey (43). A study on abattoir workers in Kazeroon city, Fars province of Iran, revealed 11.76% positivity for brucellosis based on RBT and tube agglutination techniques (44), which complies with our result (13.33%). Other studies also showed brucellosis prevalence rates of 18.52% in Pakistan (45), 16% in Argentina (46), and 4.54% in Brazil (47) using RBT. Our result complies with the report from Argentina. In a study that evaluated the brucellosis sero-prevalence in South Africa, 12.6% and 17.5% of the abattoir workers were positive using RBT and ELISA, respectively (48). In Uganda, the occurrence of anti-*Brucella* antibodies among slaughterhouse workers was 9.0% (95% CI: 6.3–12.7) using RBT (49).

The questionnaire variables had no significant effect on the results of the serological brucellosis tests. The only significant relationship was found between the abattoir staff members' declaration of getting brucellosis and the positive RBT ($P<0.05$). RBT is a preferable screening test. Although it has high sensitivity, further confirmatory tests are required to diagnose brucellosis (50). In this study, due to the high sensitivity of RBT, a relationship was observed between positive RBT cases and the history of getting brucellosis. However, no relationship was found between the serological tests and other questionnaire variables (age, education, contact with

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aborted fetuses, and consumption of unpasteurized dairy products) **(**p>0.05**).** This may be due to the relatively small number of collected samples and especially small number of positive ones. The occupational risk factor is considerable for the *Brucella*-positive result, and is similar to that in the Karimi's et al. study (34). According to world studies, slaughterhouse workers and butchers are the second high-risk group for brucellosis after livestock workers. Contact with infected ruminants' materials, including carcasses, visceral organs, feces, and blood, and inhalation of infected aerosols are considered the most important risk factors for the disease (9). All workers lacked proper protective equipment (such as gloves, masks, goggles, and boots) in this study. A study from Nigeria revealed a significant relationship between brucellosis and the lack of using personal protective equipment (51). Low sample sizes and the lack of applying other techniques in *Brucella* diagnosis such as molecular and ELISA tests were among the limitations of the study.

Conclusion

This study highlighted the practical application of serological tests, including RBT, Wright, and 2-ME as a simple strategy to monitor brucellosis and to diagnose and treat the active form of the disease in endemic regions. Although only a small frequency of the disease was found, it could cause significant health and economic damage to humans and animals in endemic areas. Monitoring high-risk occupational groups is imperative to control the disease effectively. Furthermore, the use of enough protective measures is highly recommended for slaughterhouse workers to prevent human brucellosis. More comprehensive prevalence studies on livestock are also recommended to control this zoonotic disease in rural areas.

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Conflict of Interest

The authors declare no conflicts of interest.

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Ethical Considerations

All procedures performed in studies involving human participants were in accordance with the ethical standards of Shiraz University, Iran (IACUC no: 1399/63). Written consent was obtained from all of the participants.

Code of Ethics

IACUC no: 1399/63

Authors' Contributions

AZ: data collection, methodology, MM: conceptualization, supervision, data analysis, writing, and editing. SSS: data analysis, reviewing, and editing. All authors read and approved the final manuscript.

Data Availability Statement

The data are available on request from the authors.

List of Abbreviations

Rose Bengal test (RBT)
2-mercaptoethanol (2-ME)
Immunoglobulin (Ig)
Phosphate buffered saline (PBS)
Enzyme-Linked Immunosorbent Assay (ELISA)
Statistical Package for the Social Sciences (SPSS)

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