Prevalence and risk factors associated with *Brucella abortus* and *Brucella melitensis* human infections in Sistan va Baluchistan province, southwest of Iran

Morteza Salari [1], Mohammad Reza Mahzounieh [1], Azizollah Ebrahimi [1], Niloofar Kianfar [2], Mojtaba Memariani [*, 3]

[1]Department of Pathobiology, Institute of Human and Animals zoonotic diseases, Shahrekord University, Shahrekord, Iran; [2]Department of Microbiology, Islamic Azad University, Kurdistan, Iran; [3]Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Corresponding author:** Mojtaba Memariani, Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: m.memariani@sbums.ac.ir*

ABSTRACT

Brucella spp., causes zoonotic infections. Our objective was screening and risk factors of *Brucella* species among human sera samples in Sistan va Baluchistan province of Iran. Fifty three sera samples were positive using indirect Enzyme Linked Immunosorbent Assay (ELISA), but the Wright and 2-ME agglutination tests yielded lower positive cases. Thirty one (58.5 %) samples contained *Brucella spp* using PCR among which *B. abortus* and *B. melitensis* were detected in 25 (80.6 %) and 6 (19.4 %) of specimens, respectively. Risk factors associated with *B. abortus* and *B. melitensis* infection included age ranging 25-45 years (23/31), male sex (26/31) and consumption of dairy products (22/31). Consumption of unpasteurized cow milk and livestock exposure were the significant risk factors.

Keywords: Human Brucellosis, Sistan va Baluchistan province, risk factors, Iran

INTRODUCTION

The brucellosis or Malta fever caused by *Brucella* species is one of the most common infections in humans and animals causing significant healthcare problems and economic losses [1]. The disease is caused by four species of *Brucella* in humans, and the main reason for the transmission of this bacterium is usually the use of non-pasteurized dairy products,

including cheese, ice cream, and milk and other products [2]. Additionally, transmission occurs via contact with contaminated traps or microorganisms in some occupations, including laboratory staff and veterinarians, and in some sources, blood transfusion and sexual contact have also been mentioned as transmission factors [3]. After infection, the bacterium causes non-specific clinical manifestations such as fever of unknown origin, lethargy, night sweats, and fatigue and weight loss [4,5]. If the agent be identified and eradicated for up to a month, incomplete elimination leads to chronic and disseminated infection into the whole body and appearance of other symptoms such as osteoarthritis, genital urogenital complications, meningitis, and liver and heart disease [6].

Brucella app has not been eradicated in Iran and the disease has currently remained as zoonotic infection [7,8]. There have been some reports of it from several other areas of the world [9,10]. Application of rapid and sensitive methods is helpful for the species identification and facilitates proper control measures. In addition, recognizing significant and prominent risk factors is critical for hindering its spread. It is worth considering that there is no validated and useful vaccine for human use [11, 12]. The

purpose of this study was to identify the *Brucella* species in the sera samples of infected patients in Sistan va Baluchistan province using phenotypic tests and PCR technique.

MATERIALS AND METHODS

Samples

This cross-sectional and descriptive study was conducted on 11596 suspected cases of brucellosis referred to Infectious Diseases Laboratory of Sistan va Baluchistan Province for one year from November 2015 to November 2016.

Serological tests

In the first step, slide agglutination technique was used for screening suspicious samples [1]. In order to determine the antibody titer, the WT and 2ME method was used. For the slide agglutination test, the Rose Bengal antigens were prepared from the Razi serum and vaccine research institute, and the test protocol was performed according to the instructions. The Wright and 2ME tests were performed according to the Wright test kit protocols (product antigen from

Bein Parvar Parsian Company) using positive control.

Indirect Enzyme linked immunosorbent assay

Serum samples were assessed for the existence of *Brucella spp* using specific antibodies according to the manufacturer's instructions (SVANOVA® Brucella-Ab I-ELISA SvanovBiotech AB-Uppsala). Brucella-positive serum was also used as positive control in all the assays.

DNA extraction

DNA was extracted from the sera samples of seropositive patients. The DNA extraction was performed according to the protocol introduced by Gene All cell SV mini, Korea.

PCR detection of Brucella species

The PCR assay was employed using the specific primer pairs (TAG, Copenhagen). The nucleotide sequence characteristics of the primers have been displayed in Table 1 [12]. Firstly, a universal primer was used for the *Brucella* genus amplifying a 450 bp DNA fragment for all species of *Brucella*, but not for *B. pinnipedia* and *B. ceti*, two species isolated from marine mammals. In addition, a primer with a product of 1071 bp size was employed to detect *B. abortus*, and its vaccine strains such as S19 and RB51. Another primer pair for detection of *B. Melitensis* amplifying a 218bp product was used.

Table1. The specific primers used in this study

primer	gene	Sequence: 5' to 3'	PCR product (bp)
Brucella spp	$BMI0535f$	$F: GCGCATTCTTCGGTTATGAA$	450
	$BMI0536r$	$R: CGCAGGCGAAAACAGCTATAA$	

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B. abortus	$BMEII0843f$	$F: TTTACACAGGCAATCCAGCA$	1071
	$BMEII0844r$	$R: GCGTCCAGTTGTTGTTGATG$	
B. melitensis	$BMEI0752f$	$F: CAGGCAACACCCTCAGAAGC$	218
	$BMEI0752r$	$R: GATGTGGTAACGCACACCAA$	

The PCR reaction was performed in a volume of 25 μL using the Master Mix 2 X Amplicon product from TAG, Copenhagen. The thermal cycler temperature program (Applied Biosystem, USA) was as follows: Initial denaturation of 94 °C for 3 min followed by 38 cycles including denaturation at 94 °C for 1 min, annealing at 55 °C for 45 sec, extension step at 72 °C for 1 min, and finally at 72 °C for 5 min. The PCR product was evaluated at a volume of 5uL in 1 % agarose gel wells in TAE buffer and electrophoresis at 100 volts for 1 hour. The results were recorded in the gel documentation device (UVitec, UK).

Data analysis

Data was analyzed with SPSS version 21. ANOVA and student t-test were used for this purpose considering confidence interval of 95 percent and p value <0.05 as significant result.

RESULTS

In this study, 11596 patients with age range of 4-65 years (mean age=26.4±3) with both males 7915 (68.3 %) and females 3681 (31.7 %) were evaluated. Fifty-three (3.91 %) of 11596 sera specimens were positive in the serologic method (indirect ELISA). Of these 53 positive cases, 31 cases were positive in PCR including six positive for *B. melitensis* and 25 cases for *B. abortus* species. Furthermore, 22 seropositive samples were negative in the PCR reaction.

A majority of positive patients aged between 21 and 40 years and were male highlighting the infection as a zoonotic and occupational disease. Risk factors associated with *B. abortus* and *B. melitensis* infection included age ranging 21-40 years (23/31), male sex (26/31) and

consumption of dairy products (22/31) (p<0.05) (Table 2).

Table 2. The demographic data and antibody titer in 11596 suspected patients with brucellosis

Demographic data		No. (%)	Positive with PCR No (%)	Odds ratio	95 % Cl	ρ value
Age	0-10	155(1.3)	-	Baseline		
	11-20	912(7.9)	-	2.119	1.321-3.398	**0.002**
	21-30	2527(21.8)	11 (0.0009)	4.511	2.864-7.104	**<0.0001**
	31-40	2817(24.3)	14 (0.0012)	6.609	4.190-10.424	**<0.0001**
	41-50	2435(21)	4 (0.0003)	7.263	4.589-11.495	**<0.0001**
	51-60	1623(14)	2 (0.0001)	7.33	4.582-11.728	**<0.0001**
	61<	1127(9.7)	-	5.153	3.201-8.296	**<0.0001**
Sex	Male	7915(68.3)	26 (83.87)	0.982	0.908-1.062	**0.653**
	Female	3681(31.7)	5 (16.12)			
Total		11596 (100)				

DISCUSSION

Brucellosis is one of the most common diseases in humans and livestock with symptoms such as fever and chills in humans, infertility, persistent infection, and animal health hazards. After about 50 years of struggle to fight and eradicate the disease, the disease has not been completely eliminated in most of countries, although being an accidental infection to human [13]. Determining the species of this bacterium plays an important role in identifying the reservoir and subsequently control and eradication of the infection. In this research, which was implemented on 11596 sera samples in Sistan va Baluchistan province, 53 cases (3.91 %) had a positive serologic result. Thirty-one cases (58.5 %) of them were positive in the PCR assay which the antibody against bacterium was detectable. The reason for this was probably the false positive result in the serology method. In old-time brucellosis infections, cases of rapid onset or cross-reactivity due to infection with bacteria which share common antigens occur with those in the brucellosis. Of the 31 cases infected with *Brucella spp*, 25 samples (80.6 %) were contaminated with *B. abortus* and only five samples were positive for *B. melitensis*. The results are contrary to the prevalence pattern of species in other areas of the country, because of the presence of the dominant *B. melitensis*, while herein *B. abortus* was the predominant species. There is a possibility that the most dairy products used in Sistan va Baluchistan province were from large livestock products such as cattle or contact with them. There have been reports of the infection among livestock in the province and other areas [14,15]. This is in contrary with some regions where the disease has been eradicated [16]. However, the existence of the agents is a concern which threats human health even when exported to other areas mostly through consumption of dairy or meat products [17].

In this study, risk factors associated with *B. abortus* and *B. melitensis* infection included age ranging 25-45 years (23/31), male sex (26/31) and consumption of dairy products (22/31) (p<0.05). In has been stated in some reports that *B. melitensis* has

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caused the majority of livestock and human infections [18,19], while some have concluded that *B. abortus* was the predominant species. Even in cleared areas the infection has remained among wildlife and thus there is a need to follow up and control the animals to prevent the disease.

CONCLUSION

B. abortus have remained as serious and threatening infection in some areas of the world. Animals are reservoirs of the species. In this study, *B. abortus* was predominant species. Risk factors associated with *B. abortus* and *B. melitensis* infection included age, genus, and livestock exposure, consumption of milk, yoghurt, unprocessed cheese and ice cream ($p<0.05$). Therefore, accurate planning to control the contamination in livestock; especially cows populations in the province and to prevent the supply of non-sanitary livestock products seems essential.

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