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Prevalence of proline racemase/ hydroxyproline epimerase gene in human *brucella* isolates in Iran

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Abstract

Background: Human brucellosis is a zoonotic disease caused by *Brucella melitensis*, *Brucella abortus,* and *Brucella suis*. *Brucella* causes a chronic disease, which subverts the immune defense system of their hosts. In this study, the prevalence of an important *Brucella* virulence determinant, PrpA, which can modulate immune response, was determined in human isolates.
Methods: Polymerase chain reaction (PCR) assay was standardized and applied to 37 isolates obtained from patient's specimens. Primers for *prpA* gene were designed and evaluated using bioinformatic tools. DNA sequencing was performed for further verification.
Results: In the 37 *Brucella* isolates (31 *Brucella melitensis* and 6 *Brucella abortus*), 32 (86.4%) carried *prpA* gene.
Conclusion: Presence of *prpA* gene in most isolates indicates the high prevalence of this gene among Iranian isolates and emphasizes its role in pathogenicity of this organism.

Keywords: *Brucella* species, Brucellosis, Proline Racemase, PrpA

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Introduction

Brucella, a Gram negative coccobacillus, is responsible for brucellosis disease in domestic and wild animals and is transmittable to human hosts. Although humans are not the main sources of infection, the disease causes socioeconomic problems in many countries (1). Broad spectrum of mammalians is at risk of becoming infected with *Brucella*. Humans are mainly infected by *Brucella melitensis*, *Brucella abortus,* and *Brucella suis* (2-3). Human brucellosis is transmitted through several routs such as ingestion of contaminated dairy products, inhalation, and direct contact with infected animal tissues; however, person to person transmission is extremely rare (4-5). The disease is febrile and debilitating for human hosts, with particular problems and constitutional symptoms (6). Additionally, brucellosis is a chronic and persistent infection with a capacity of becoming granulomatous (7).

Pathogenicity in *Brucella* and other intracellular organisms such as *Leishmania* spp., *Trypanosomes* and *Salmonella typhi* depends on survival and replication of the organism inside the host cell. These pathogens develop multiple approaches to subvert the host immune responses. The ability to hide and survive in host cells leads to establishment of a chronic infection (8-9). Accordingly, *Brucella* utilizes numerous factors such as type IV secretion system (VirB), cyclic β1, 2-glucans, and LPS to manipulate the host's immune system. Identification of new putative factors has opened many doors for better understanding of pathogenicity (10).

Brucella PrpA (Proline Racemase Protein A), homologous to a proline racemase with mitogenic activity in the human protozoan parasite *Trypanosoma cruzi*, is a T-independent B lymphocyte mitogen and a potent IL-10 inducer required for the establishment of chronicity and the early immune suppression observed in mice after infection (10). PrpA uses NMM-IIA (nonmuscular myosin IIA) for attachment to macrophages to activate lymphoproliferation (11). Although PrpA has been described as immunomodulatory molecule in several pathogens (12-14), there had been no information on the prevalence of PrpA in *Brucella* species in Iran. In the present study, a

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↑What is "already known" in this topic:
PrpA as an immunomdulatory molecule activates IL-10 and stimulates B cell replication. Organism such as *Brucella* requires PrpA to establish chronic infections.

→What this article adds:
High prevalence of *prpA* gene in human *Brucella* isolates may increase the virulence capacity among Iranian isolates of *B. melitensis* and *B. abortus.*

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Table 1. PCR primer sequence used for amplification of the Brucella prpA gene.

Target gene	Primer designation	Oligonucleotide sequence (5'-3')	PCR product (bp)
PrpA	PrpA-F	AACCTCAATGGATCGACC	672
	PrpA-R	ACGGTCGATAGCCTTGTC	

PCR assay was developed to detect *prpA* gene that was applied to 37 *B. melitensis* and *B. abortus* isolates obtained from patients in cities of Tehran, Arak and Hamadan in Iran.

Methods

Bacterial strains

From a total of 37 isolates, 31 *B. melitensis* and 6 *B. abortus* were identified based on bacteriological tests such as colony morphology, Gram staining, oxidase, catalase, CO_2 growth, H_2S production, and dye tolerance such as basic fushin and thionin.

DNA preparation

A loopful of colonies of each isolate on *Brucella* agar plates was picked and suspended in 200 µL of distilled water. After vortexing, the suspension was boiled for 5 minutes, and 50 µL of the supernatant was collected after spinning at 14 000 rpm for 10 minutes. The DNA concentration of the boiled extracts was determined with a spectrophotometer.

PCR assay

PCR amplifications were performed in a final volume of 25 µL in PCR tubes. The reaction mixtures consisted of 2 µL of the DNA template, 1 µL of each primer, 8 µL of mastermix (Taq DNA polymerase Mastermix Mix Red, $MgCl_2$, Amplicon), and the total volume was adjusted to 25 µL using distilled deionized water. PCR program for amplification of *prpA* consisted of initial denaturation at 94°C for 4 minutes, 30 cycles of application with denaturation at 94°C for 1minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. Electrophoresis of PCR products was performed on 1.5 % agarose gels using 100-bp DNA ladder as molecular size marker. Gels were visualized under UV and documented using Uvitec System DOC-008.XD (EEC).

Primer designation and DNA sequencing

The nucleotide sequence of *Brucella prpA* gene was obtained from *B. melitensis* 16M chromosome II complete sequence (AE008918.1) in NCBI GenBank database. Specific primer set was designed based on pairwise and multiple sequence alignment for the corresponding gene using CLC Sequence Viewer Version 6.8.2. The primer sequences and their amplicon size are demonstrated in Table 1.

Table 2. Demographic characteristics of human Brucella isolates.

NUMBER	*GENDER*	*SPECIMEN TYPE*	BRUCELLA *SPECIES*	*STRAIN*	*PROVINCE/CITY*
1	*Female*	*CSF*	B. melitensis	*1-A*	*Arak*
2	*Female*	*CSF*	B. melitensis	*2-A*	*Arak*
3	*Male*	*Blood*	B. melitensis	*3-A*	*Arak*
4	*Male*	*Blood*	B. melitensis	*4-A*	*Arak*
5	*Female*	*Blood*	B. abortus	*5-A*	*Arak*
6	*Male*	*CSF*	B. melitensis	*12-A*	*Arak*
7	*Male*	*Blood*	B. abortus	*16-A*	*Arak*
8	*Male*	*Blood*	B. melitensis	*19-A*	*Arak*
9	*Male*	*Blood*	B. melitensis	*20-A*	*Arak*
10	*Female*	*Blood*	B. melitensis	*18-H*	*Hamadan*
11	*Male*	*Blood*	B. melitensis	*22-H*	*Hamadan*
12	*Female*	*Blood*	B. melitensis	*33-H*	*Hamadan*
13	*Male*	*Blood*	B. melitensis	*34-H*	*Hamadan*
14	*Male*	*Blood*	B. melitensis	*35-H*	*Hamadan*
15	*Female*	*Blood*	B. melitensis	*39-H*	*Hamadan*
16	*Male*	*Blood*	B. melitensis	*40-H*	*Hamadan*
17	*Male*	*Blood*	B. abortus	*46-H*	*Hamadan*
18	*Male*	*Blood*	B. melitensis	*48-H*	*Hamadan*
19	*Male*	*Blood*	B. melitensis	*54-H*	*Hamadan*
20	*Male*	*Blood*	B. melitensis	*1-T*	*Tehran*
21	*Female*	*Blood*	B. melitensis	*2-T*	*Tehran*
22	*Female*	*Blood*	B. abortus	*3-T*	*Tehran*
23	*Female*	*Blood*	B. melitensis	*5-T*	*Tehran*
24	*Male*	*CSF*	B. abortus	*11-T*	*Tehran*
25	*Female*	*Blood*	B. abortus	*18-T*	*Tehran*
26	*Male*	*Blood*	B. melitensis	*19-T*	*Tehran*
27	*Male*	*Blood*	B. melitensis	*21-T*	*Tehran*
28	*Female*	*Blood*	B. melitensis	*22-T*	*Tehran*
29	*Male*	*CSF*	B. melitensis	*24-T*	*Tehran*
30	*Male*	*Blood*	B. melitensis	*27-T*	*Tehran*
31	*Male*	*Blood*	B. melitensis	*29-T*	*Tehran*
32	*Male*	*Blood*	B. melitensis	*30-T*	*Tehran*
33	*Female*	*Blood*	B. melitensis	*31-T*	*Tehran*
34	*Female*	*CSF*	B. melitensis	*32-T*	*Tehran*
35	*Female*	*CSF*	B. melitensis	*33-T*	*Tehran*
36	*Male*	*CSF*	B. melitensis	*385-T*	*Tehran*
37	*Male*	*Blood*	B. melitensis	*14508-T*	*Tehran*

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Table 3. Distribution prpA genes among 37 human Brucella isolates.

Virulence gene	Human isolates (n=37)	
	B. melitensis (n=31)	B. abortus (n=6)
prpA	31 (100)	1 (16.7)

The amplified fragment of *prpA* gene was purified using Silica Bead DNA Gel Extraction Kit (Thermo Scientific, USA). Sequencing was performed on both strands using an automated sequencer system (ABI 3730xl DNA Analyzer). DNA sequences were edited by Chromas Lite Version 2.5.1 (Technelysium Pty Ltd, Australia) and BioEdit Version 7.2.5 (Hall, 1999).

Results

From a total of 37 isolates, 31(83.7%) *B. meltensis* and 6 (16.3) *B. abortus* were identified from 29 blood and 8 CSF human samples. Of the patients, 23 (62%) were male and 14 (38%) were female. Detailed information on specimen type, *Brucella* species type, and areas of sample collection are presented in Table 2. As expected, *prpA* gene assay produced an amplicon of 672 bp (Fig. 1).

In the current study, all *B. melitensis* isolates had *prpA* gene; however, just one of the six *B. abortus* isolates was positive for this gene (Table 3). Analysis of the sequenced gene with Chromas software and blast in the NCBI (National Center for Biotechnology Information) site showed the same DNA sequences; therefore, all the PCR assay results were confirmed.

Discussion

Brucella has an amazing ability to adapt to hosts' cellular atmosphere and evade immune responses. Other organisms which cause chronic diseases such as *Mycobacteium tuberculosis*, *Salmonella* spp, and *Trypanosoma cruzi* use the same mechanism to reach their replication niche (15). Although little is known about the molecular mechanisms of *Brucella* virulence factors, numerous putative factors have been identified (16).

The present study aimed at identifying *B. melitensis* and *B. abortus* strains, which were carriers of *prpA* gene. Our results confirmed the presence of *prpA* gene in 32 (86.4%) *B. melitensis* and *B. abortus* isolates collected from human patient specimens. Interestingly, 5 out of 6 *B. abortus* isolates lacked this gene, which is not completely consistent with the result of Spera et al. (10). Our findings revealed the presence of *prpA* gene in human isolates of *Brucella* (100% *B. melitensis*, and 16.6% *B. abortus*)and not in animal isolates, which may be responsible for variations in results. It can be concluded that the present gene is more frequent in *B. melitensis* strains compared with *B. abortus* strains in Iran; however, there is no warrant for expression of the gene.

Proline racemase protein, a homodimeric enzyme, was initially identified in *Clostridium sticklandii* protobacterium; however, a eukaryotic proline racemase was first isolated from *Trypanosoma cruzi*(TcPRAC), the causative agent of Chagas disease (17). According to Goytia et al., Proline racemase function is similar to hydroxy proline epimerase activity as a lymphocyte mitogen (18). This enzyme is capable of converting L and D-proline enantiomers reversibly (19); D-amino acids are often found in eukaryotes and bacterial cell walls (20). Proline racemase antibodies and inhibitor, and pyrrole-2-carboxylic acid (PYC) exert influence on the infection of *T. cruzi* in vitro. PYC can also interfere in intracellular *T. cruzi* differentiation (19). PrpA in *Brucella* is a molecular virulence factor, which can put the host's immune system in an anergic state (10).

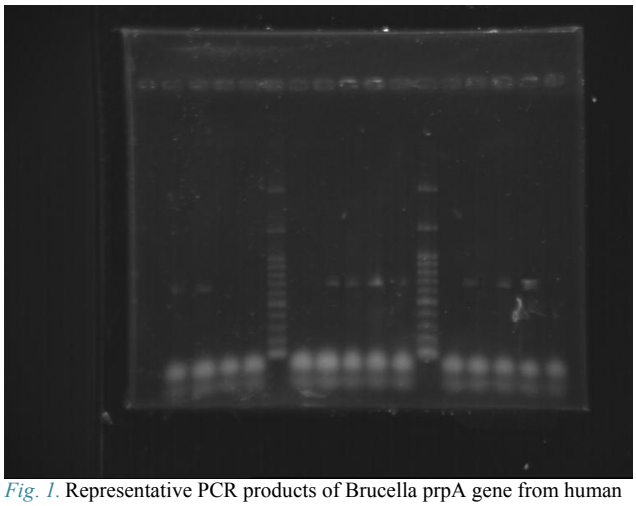

Fig. 1. Representative PCR products of Brucella prpA gene from human strains of B. melitensis and B. abortus. Lanes M, DNA Ladder Mix; Lanes 2,3,7,8,9,10,12,13 are Brucella melitensis strains positive for prpA, Lane 14 Brucella abortus strain positive for prpA, Lanes 4,5,6,11,15 are Brucella abortus strains negative for prpA.

Conclusion

The present study demonstrated a very high level of *prpA* gene presence in human *Brucella* isolates. The design of a PCR test to assess the presence of aforementioned gene was a first attempt to understand the mechanism of virulence, especially in Iranian isolates of *Brucella melitensis* and *Brucella abortus*. For further understanding of *prpA* genetic epidemiology, similar studies on animal isolates may be required.

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Conflict of Interests

The authors declare that they have no competing interests.

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