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Short Communication

Detection of *Brucella melitensis* and *Brucella abortus* strains using a single-stage PCR method

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ABSTRACT

Brucella melitensis and *Brucella abortus* are of the most important causes of brucellosis, an infectious disease which is transmitted either directly or indirectly including consuming unpasteurized dairy products. Both strains are considered endemic in Iran. Common diagnostic methods such as bacteriologic cultures are difficult and time consuming regarding the bacteria. The aim of this study was to suggest a single-stage PCR method using a pair of primers to detect both *B. melitensis* and *B. abortus*. The primers were named UF1 and UR1 and the results showed that the final size of PCR products were 84 bp and 99 bp for *B. melitensis* and *B. abortus*, respectively. Therefore the method could be useful for rapid detection of *B. melitensis* and *B. abortus* simultaneously.

Keywords: *B. melitensis*, *B. abortus*, PCR, Detection, Brucellosis

INTRODUCTION

Brucella is a nonmotile, small, gram negative, strictly aerobic coccobacilli. It is mostly positive in catalase and oxidase tests and shows various results in urease tests (Young 1995). *Brucella* spp. genome studies indicate more than 70 % homology (Clavareau *et al* 1998), so DNA-DNA hybridization proposed *Brucella melitensis*is as the only species and others as its biovars (Verger *et al* 1985). *B. melitensis* and *B. abortus* are causative agents of small ruminant and bovine brucellosis, respectively (Al-Ani *et al* 2004). The most significant clinical signs of brucellosis in animals are orchitis and epididymitis in males and abortion, reproductive disorders and placenta retention in females (Refai 2002). The outbreaks of bovine brucellosis generally occur with abortion during the last 3 months of pregnancy and result in weak calves and infertility in cows (Fekete *et al* 1992). Brucellosis incidences in humans are mostly resulted from contacting with infected animals or consuming non-pasteurized dairy products (Young 1995), with Mediterranean, Middle Eastern and Latin American areas as high risk areas (Arnow *et al* 1984, Gedikoglu *et al* 1996, Yagupsky *et al* 1994, Young 1995). The bacterium causes febrile septicemia, localized infection

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of bone and different organs of humans with varying incubation period (Young 1995). Besides, various reports are published in where *B. melitensis* is endemic. Number of abortions without any clinical signs has been increased among the residents of these regions (Boschiroli *et al* 2001). Due to higher specificity of bacteriological testing, it is the most accurate method to confirm brucellosis. Various *Brucella* biotypes of its species could help to find out the infection source (Guler *et al* 2003). On the other hand, classical methods such as culture-based methods are time consuming and need practice. PCR with various target genes and different primers have been used to diagnose brucellosis and to identify *Brucella* species (Cogswell *et al* 1996, Thomsen *et al* 1999, Fekete *et al* 1990, Baily *et al* 1992, Herman *et al* 1992, Romero *et al* 1995, Leal-Klevezas *et al* 1995). As it is expected, PCR-based methods are faster and more sensitive than traditional methods but the sensitivity and specificity vary in laboratories (Navarro *et al* 2004). Since sheep and goat dairy products are used in rural of Iran, brucellosis is endemic in these areas. Detecting brucellosis is critical for epidemiological and preventative objectives in animals and humans. The aim of this study was to evaluate a single-stage PCR method to differentiate *B. melitensis* and *B. abortus* and to compare the results using bacteriological methods. We have used a pair of primers to detect both species with different PCR product weights.

MATERIALS AND METHODS

Bacterial strains. To do the study, 41 *Brucella* strains including *B. melitensis* (n: 28) and *B. abortus* (n: 13) were studied. Also *B. melitensis* 16M (ATCC 23456; NCTC 10094), *B. abortus* 544 (ATCC 23448; NCTC 10093), *B. suis* 1330 (ATCC 23444; NCTC 10316), *B. neotomae* 5K33 (ATCC 23459; NCTC 10084) standard strains were used as controls. Whole genomic DNA of all *Brucella* isolates were extracted by high pure PCR template preparation kit (Roche), after culturing on *Brucella* agar. The DNA volumes of the samples were assessed and purified to 30-60 ng/ml.

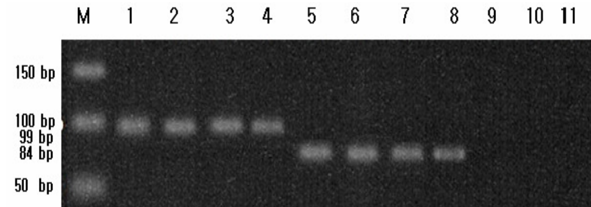
Comparative genomic analysis and primer designing. The whole sequence of *Brucella* chromosome 1 was analyzed and compared with all other chromosomes and available standard *Brucella* strains. Only one locus was found with the capacity of designing a proper primer to differentiate *B. melitansis* and *B. abortus*. The primers were designed by Oligo Software Version 5.

PCR assay. In this study, used primers to detect the B. *melitensis* and *B. abortus* were called UF1 and UR1. Using NCBI sequences, The primers were designed in a way that the target sequence covers all intra-species biovars. Therefore, specific loci were considered as *B. abortus* and *B. melitensis* (Table 1). Also specific primers were designed to compare our method with classic methods in *Brucella* detection. PCR was performed to all standard and Iranian isolates using specific primers. PCR mix for *B. abortus* and *B. melitensis* detection by UF1 and UR1 included 50 ng DNA, 2.5 mM MgCl2, 0.4 µM of each primers UF1 (5'-GGCTATCGGCTGGGAAAGG-3') and UR1 (5'-CCTTCCGAAGAAAATACCCCT-3'), 1.25 U of *Taq* DNA Polymerase, 200 µM dNTP diluted 1X PCR buffer and sterile distilled water up to 25 µl volume . The thermal cycles were adjusted to 30 95°C cycles after initial denaturation for four minutes, 30 seconds at 94°C, 30 seconds at 52°C and 45 seconds at 72°C. Finally, a final extension step was performed at 72°C for 5 minutes. Electrophoresis was performed using 3% molecular grade agarose gel (Cinaclone, Iran) at 75v for 120 minutes.

RESULTS AND DISCUSSION

PCR results regarding the standard strains of *B. melitensis* and *B. abortus* were specific while no unspecific reactions were seen with *B. suis* and *B. neotomae* (Figure 1). Results of native strains of *B. melitensis* and *B. abortus* were similar to the standard ones. The final weights of PCR products using UF1 and UR1 primers were 84 bp and 99 bp for *B. melitensis* and *B. abortus*, respectively. The incidence of human brucellosis is directly related to the level of

animal brucellosis in specific regions (Godfroid *et al* 2005). The real rate of human brucellosis is estimated to be 10 to 25 times more than annual reports (Corbel 1992). During the recent decades, many preventative strategies were performed on small ruminants. However, brucellosis is still endemic in most Mediterranean and Middle Eastern countries (Refai 2002). In these countries, milking goats and sheep is mostly done by hands; Also rural people can be indirectly and directly contacted with infected excretions especially in reproduction seasons (Minas *et al* 2007). Detecting *Brucella* species from infected animals and humans has been a public health issue for establishing control and preventative strategies in a region. Based on our results, the single-stage PCR using UF1 and UR1 primers could detect standard and native strains of *B. meliensis* and *B. abortus*. Diagnosis of human brucellosis in Iran is mostly based on culture (Yagupsky 1999) and serologic tests (Alton *et al* 1975) but PCR-based diagnostic methods are rarely used in developing countries (Khosravi *et al* 2006). Even though some techniques such as Buffered *Brucella* Antigen, Serum Agglutination Test and ELISA could diagnose brucellosis, they could not detect the causative species (Nagalingam *et al* 2012). Recently due to limitations of culturing and serological methods, several molecular methods such as PCR have been developed for rapid diagnosis of brucellosis (Kamal *et al* 2013). PCR is a cost benefit, rapid, simple, sensitive and specific method for detection of *Brucella* species (Kamal *et al* 2013). Several PCR based methods are presented for *Brucella* diagnosis all over the world. In the recent studies, some primers were designed to detect all intra-species biovars of *B. melitensis* and *B. abortus* (Fekete *et al* 1990, Baily *et al* 1992, Herman *et al* 1992, Romero *et al* 1995, Leal-Klevezas *et al* 1995). Based on the new genomic sequences of *Brucella* isolates discovered since 2009 and bioinformatics analysis, it seems that the introduced primers of these studies do not have enough efficacies to detect all intra-species biovars. Mean while in this study, two primers were developed to detect *B. melitensis* and *B. abortus* simultaneously.



Figuer 1. M: DNA Ladder, 50bp-plus, Lane1: *B. abortus* 544 (ATCC 23448; NCTC 10093) 99 bp, Lane 2 to 4: *Brucella abortus* (native strains), Lane 5: *B. melitensis* 16M (ATCC 23456; NCTC 10094) 84 bp,Lane 6 to 8: *Brucella melitensis* (native strains), Lane9: *B. suis* 1330 (ATCC 23444; NCTC 10316), Lane10: *B. neotomae* 5K33 (ATCC; NCTC 10084), Lane 11 Control Negative.

Table 1. Primers position in complete sequence of *B. abortus* and *B. melitensis* chromosome 1

Brucella species	Primers	Primer position	Target	Product size
Brucella abortus A13334	Uf1	1459361-1459379	Hypothetical protein, conserved	99 bp
	Ur1	1459440-1459459		
Brucella abortus S19	Uf1	957047-957065		
	Ur1	956967-956987		
Brucella abortus biovar 1 str. 9-941	Uf1	958748-958766		
	Ur1	958668-958688		
Brucella melitensis bv. 1 str. 16M	Uf1	1048562-1048580		84 bp
	Ur1	1048625-1048645		
Brucella melitensis M5-90	Uf1	958796-958814		
	Ur1	958731-958751		
Brucella melitensis M28	Uf1	958482-958500		
	Ur1	958417-958437		
Brucella melitensis ATCC 23457	Uf1	958538-958556		
	Ur1	958473-958493		

The genomic locus was found by comparative genomic analysis of complete sequence of chromosome I with all deposited sequences in the gene bank. PCR results of native *B. melitensis* and *B.*

abortus strains were similar to standard strains. Our primers produced the products with different sizes: 84bp and 99bp for *B. melitensis* and *B. abortus*. In conclusion, this PCR method could ease and accelerate the process of diagnosis and detection of *Brucella* species. In this study, the designed primers were able to detect both species (*B. melitensis* and *B. abortus*) and differentiate them from other *Brucella* species.

Ethics

I hereby declare all ethical standards have been respected in preparation of the article.

Conflict of Interest

Hereby, I declare "no conflict of interest exists" regarding submitted article.

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