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* 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 (Boschirolí 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. melitensis 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 developed to detect B. melitensis and B. abortus simultaneously.

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

<table>
<thead>
<tr>
<th>Brucella species</th>
<th>Primers</th>
<th>Primer position</th>
<th>Target</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. abortus</td>
<td>UF1</td>
<td>1459361-1459379</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UM1</td>
<td>1459440-1459459</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. abortus S19</td>
<td>UF1</td>
<td>957047-957065</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UM1</td>
<td>956967-956987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. abortus biovar 1 str. 9-941</td>
<td>UF1</td>
<td>958748-958766</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UM1</td>
<td>958668-958688</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. melitensis bv. 1 str. 16M</td>
<td>UF1</td>
<td>1048562-1048580</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UM1</td>
<td>1048625-1048645</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. melitensis M5-90</td>
<td>UF1</td>
<td>958796-958814</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>UM1</td>
<td>958731-958751</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. melitensis M28</td>
<td>UF1</td>
<td>958482-958500</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UM1</td>
<td>958417-958437</td>
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</tr>
<tr>
<td>B. melitensis ATCC 23457</td>
<td>UF1</td>
<td>958388-958556</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UM1</td>
<td>958473-958493</td>
<td></td>
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</tr>
</tbody>
</table>

The genomic locus was found by comparative genomic analysis of complete sequence of chromosome 1 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.

References


