Detection and Segregation of *Brucella abortus* and *Brucella melitensis* in Aborted Bovine, Ovine, Caprine, Buffaloes and Camelid Fetuses by Application of Conventional and Real-time Polymerase Chain Reaction

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Abstract

This present study was carried out to use conventional and real-time PCR for detection and segregation of *Brucella abortus* and *Brucella melitensis* in aborted bovine, ovine, caprine, buffalo and camel fetuses. All samples were collected and immediately transferred to laboratory, genomic DNA was extracted and the conventional and real-time PCR by specific primers for *Brucella abortus* and *Brucella melitensis* was performed. A TaqMan analysis and single-step PCR was carried out in total 3710 DNA of abomasal contents of aborted fetuses. In total, 281/892 (31.5%) bovine, 224/810 (27.65%) ovine, 219/786 (27.86%) caprine, 199/604 (32.94%) buffalo and 201/618 (32.52%) camel fetus samples gave positive results for *Brucella* species by conventional PCR. Moreover, 45/281 and 231/281, 169/224 and 49/224, 194/219 and 22/219, 57/199 and 137/199 and finally 51/201 and 143/201 specimens were positive for *B. melitensis* and *B. abortus* in aborted bovine, ovine, caprine, buffalo and camel fetuses by real-time PCR, respectively. The sensitivity and specificity of real-time PCR obtained 100% and 100%. Statistical analysis showed significant differences ($p<0.01$) between *B. abortus* and *B. melitensis* that were detected in abomasal contents of aborted bovine, ovine, caprine, buffalo and camelid fetuses and between presences of *Brucella* spp. in bovine with caprine, buffalo and camel aborted fetuses ($p<0.05$). The CT values obtained from real-time PCR had significant differences between aborted bovine, ovine, caprine, buffalo and camel fetuses for presence of *B. abortus* and *B. melitensis*. Results showed that the real-time PCR is considerably faster than current standard methods for isolation and segregation of *Brucella* spp.

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บทคัดย่อ

การตรวจหาและแยกเชื้อ *Brucella abortus* และ *Brucella melitensis* ในตัวอ่อนแท้งของโค แกะ แพะ กระบือ และอูฐ โดยปฏิกิริยาพีซีอาร์ แบบ conventional และ real time

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การศึกษานี้ใช้ปฏิกิริยาพีซีอาร์ แบบ conventional และ real time ในการตรวจสอบและคัดแยกเชื้อ *Brucella abortus* และ *Brucella melitensis* ในตัวอ่อนแท้งของโค แกะ แพะ กระบือ และอูฐ ตัวอย่างทั้งหมดถูกเก็บและขนส่งไปยังห้องปฏิบัติการทันที เพื่อทำการสกัดจีโนมิกดีเอ็นเอ และตรวจด้วยปฏิกิริยาพีซีอาร์ แบบ conventional และ real time ด้วยไพรเมอร์ที่เฉพาะเจาะจงสำหรับ *Brucella abortus* และ *Brucella melitensis* ตัวอย่างดีเอ็นเอจำนวน 3710 ตัวอย่างที่เก็บจากของเหลวในกระเพาะแท้ของตัวอ่อนที่แท้ง ทำการวิเคราะห์ด้วย TaqMan และปฏิกิริยาพีซีอาร์ แบบขั้นเดียว พบว่าจำนวนตัวอย่างที่ให้ผลบวกต่อ *Brucella* จากตัวอย่างทั้งหมดเป็น 281/892 (31.5%) ในโค 224/810 (27.65%) ในแกะ 219/786 (27.86%) ในแพะ 199/604 (32.94%) ในกระบือ และ 201/618 (32.52%) ในอูฐ เมื่อใช้ปฏิกิริยาพีซีอาร์ แบบ conventional และพบว่าตัวอย่างที่ให้ผลบวกต่อ *B. melitensis* และ *B. abortus* จากตัวอย่างทั้งหมดคิดเป็น 45/281 และ 231/281 ในโค 169/224 และ 49/224 ในแกะ 194/219 และ 22/219 ในแพะ 57/199 และ 137/199 ในกระบือ และ 51/201 และ 143/201 ในอูฐ เมื่อใช้ปฏิกิริยาพีซีอาร์ แบบ real time ความไวและความจำเพาะของ ปฏิกิริยาพีซีอาร์ แบบ real time คิดเป็น 100% และ 100% การวิเคราะห์ทางสถิติแสดงให้เห็นความแตกต่างอย่างมีนัยสำคัญ (*p*<0.01) ระหว่าง *B. abortus* และ *B. melitensis* ที่ตรวจพบในตัวอย่างที่เก็บจากของเหลวในกระเพาะแท้ของตัวอ่อนที่แท้งของโค แกะ แพะ กระบือ และอูฐ บัฟฟาโลและทารก Camelid และระหว่าง *Brucella* spp. ในโค กับแพะ กระบือ และอูฐ (*p*<0.05) ค่า CT ที่ได้จากปฏิกิริยาพีซีอาร์ แบบ real-time มีความแตกต่างทางสถิติระหว่างโค แกะ แพะ กรบือ และอูฐสำหรับการตรวจพบ *B. abortus* และ *B. melitensis* ผลการศึกษาชี้ให้เห็นว่าปฏิกิริยาพีซีอาร์ แบบ real-time ให้ผลการตรวจเร็วกว่าวิธีมาตรฐานในปัจจุบัน ในการแยกและการคัดแยกเชื้อ *Brucella* spp.

คำสำคัญ: ตัวอ่อนแท้ง *Brucella abortus Brucella melitensis* ปฏิกิริยาพีซีอาร์ แบบ conventional ปฏิกิริยาพีซีอาร์ แบบ real-time

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Introduction

Brucellosis is a worldwide highly contagious zoonotic bacterial disease of human and many species of animals that is caused by gram-negative, aerobic and facultative intracellular bacteria of the genus *Brucella*. This disease is an important public health problem in many parts of the worlds such as the Mediterranean littoral, the Middle East and parts of Latin America (Al-Mariri et al., 2001). *Brucella* species (*Brucella* spp.) are classically classified into 6 main species including *Brucella abortus (B. abortus), B. melitensis, B. suis, B. canis, B. ovis and B. neotomae* (Garin-Bastuji et al., 1998) and among these six species of *Brucella*, *B. abortus* and *B. melitensis* can cause abortion in ruminants (Megid et al., 2010).

In some countries especially in Europe and Asia (including Iran) where animals like Camelid and Buffaloes are kept in close contact with sheep, goats and cattles, infections and abortions can also be caused by *B. melitensis* and *B. abortus* (Radwan et al., 1992; Jensen et al., 1999; Renukaradhya et al., 2001). In Iran, *B. abortus* was first isolated from a bovine fetus in 1944 (Delpy and Kaveh, 1945) and *B. melitensis* was first isolated from a sheep in Isfahan province in 1952 (Kaveh, 1952) and then brucellosis has been reported from various species such as sheep (Zowghi et al., 2008), goat (Akbarmehr and Ghiyamirad, 2011), cattle (Zowghi and Ebadi, 1985), camel (Khadjeh et al., 1999), dog (Mosallanejad et al., 2009), buffalo (Nowroozi-Asl et al., 2007), human (Kazemi et al., 2008) and horse (Tahamtan et al., 2010) from different parts of Iran. In the majority of cases of brucellosis in

Iran, *B. abortus* and *B. melitensis* are the main pathogens. Brucella vary in the frequency with which they infect particular host species. Thus, *B. abortus* infects cattle and is sometimes transmitted to many other hosts and *B. melitensis* primarily infects sheep and goats and can also be transmitted to other hosts.

To reduce economic losses from brucellosis, accurate, safe and sensitive diagnostic methods play a vital role in the control and eradication of brucellosis in animals and humans. There are various assays for diagnosis of brucellosis such as culture, serological and molecular methods. Culture method requires a living host and is both time consuming and hazardous (Navarro et al., 2004). Diagnosis of brucellosis by serological responses is not recommended because it can be unspecific and subsensitive due to cross-reaction with other pathogens including *Yersinia enterocolitica, Salmonella genus, Escherichia coli* O:157 and other *Brucella* spp. (Corbel et al., 1984; Cventik et al., 2004; Nielsen et al., 2004). The usual method for detection and segregation of *Brucella* spp. is based on phenotypic traits, but it is associated with a high risk of laboratory-acquired infections and very time consuming (Navarro et al., 2004).

Therefore, in order to facilitate these problems, and in spite of the high degrees of genetic similarity of *Brucella* spp., several conventional and real-time PCR assays that are easier, faster, safer, more convenient and more accurate than traditional methods have been developed (Bricker, 2002; Scott et al., 2007; Foster et al., 2008). PCR has been developed for the detection of *Brucella* in a wide variety of clinical samples such as semen (Kim et al., 2006), blood (Queipo-Ortuño et al., 1997), milk (Rijpens et al., 1996), aborted fetuses (Buyukcangaz et al., 2011) and lymphoid tissue (Ilhan et al., 2008) and has been introduced as an accurate and sensitive assay for detection of *Brucella* spp.

Therefore, the two-fold purposes of this current study were to detect *Brucella* spp. in bovine, ovine, caprine, buffalo and camel aborted fetus samples by conventional PCR and to introduce the real-time PCR assay for detection and separation of *B. melitensis* and *B. abortus* in aborted fetuses.

Materials and Methods

Samples: In total, 892 bovine, 810 ovine, 786 caprine, 604 buffalo and 618 camel aborted fetus samples were collected randomly from 683 commercial herds in various parts of Iran during March to May of 2010. All samples had only abomasal contents of aborted fetuses which were collected under sterile hygienic conditions and were immediately transported at 4^0C to the laboratory in a cooler with ice packs. All abomasal content samples were kept at -20^0C until processing.

DNA extraction: From each animal, 10 ml of abomasal contents of aborted fetuses were collected by 21G sterile needle. DNA extraction was performed according to the method of Consuelo Vanegas et al. (2009). Purification of DNA was achieved using a genomic DNA purification kit (Fermentas, GmbH,

Germany) and the total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell (Sambrook and Russell, 2001).

Conventional PCR assay: This study used PCR primers that were designed by Leal-Klevezas et al. (1995). PCR primers that were used to screen the *Brucella* spp. detected DNA sequence of the gene coding for the outer membrane protein (omp-2) reported for Brucella in GenBank database located at NCBI (Leal-Klevezas et al., 1995). The forward primer sequence is 5'-GCGCTCAGGCTG CCGACGCAA-3', and the reverse primer sequence is 5'-ACCAGCCATTGCGGTCGGTA-3'.

The PCR reaction was performed in a total volume of 25 µl containing 10 µl of DNA concentrated in 2 µl of DNA sample, 0.5 mm $MgCl_2$, 0.2 mm dNTPMix, 0.8 µm of each primers and 0.5 U/reaction of Taq DNA polymerase. Reactions were initiated at 94^0C for 5 min, followed by 30 cycles of 94^0C for 50 sec, 65^0C for 40 sec, 72^0C for 1 min and a final elongation step at 72^0C for 5 min, with a final hold at 4^0C in a DNA thermal cycler (Master Cycler Gradiant, Eppendrof, Germany). A negative control (sterile water), and a positive control DNA from *B. abortus* strain S19 (S19 vaccine strain) (Razi Institute, Iran) were included in each amplification run. The PCR-amplified products (OMP2: 113-bp) were examined by electrophoresis (120 V/208 mA) in a 1.5% agarose gel, stained with a 1% solution of ethidium bromide, and examined under UV illumination. Brucella DNA was used to provide the positive control for both species.

Real time PCR assay: The real time PCRs for species segregation were based on unique genetic loci of *B. melitensis* and *B. abortus*. The primer set (that was designed by the author) consisted of BMEII0466 (5'-TCGCATCGGCAGTTTCAA/ CCAGCTTTTGGCCTT TTCC-3') (112bp) with the Cy5-CCTCGGCATGGC CCGCAA-BHQ-2 (5'Fluorophore→3'Quencher) internal probe and BruAb2_0168 (5'-GCACACTCA CCTTCCACAACAA/CCCCGTTCTGCACCAGACT-3') (222bp) with the FAM-TGGAACGACCTTTGCAG GCGAGATC-BHQ-1 internal probe.

A typical 25 µl reaction contained 12.5 µl TaqMan® Universal PCR Master Mix (foodproof® *Brucella* Detection Kit), a 300 nM concentration of each forward and reverse primer (BioNeer Corporation, South Korea), a 200 nM concentration of the probe (BioNeer Corporation, Republic of Korea), and 2.5 ng of sample DNA. TaqMan Master Mix Real time PCRs reactions were carried out using a RotorGene 6000 instrument (Corbett Research, Australia). The reaction mixture was initially incubated for 10 min at 95^0C. Amplification was performed for 45 cycles of denaturation at 95^0C for 20 sec, annealing and extension at 62^0C for 1 min. The foodproof® *Brucella* Internal control (White cap) and foodproof® *Brucella* Control Template (Purple cap) were used as an internal and positive control, respectively.

Data Analysis: Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp, Redmond, WA,

USA) for analysis. Using SPSS 18.0 statistical software (SPSS Inc, Chicago, IL, USA), ANOVA test analysis were performed and differences were considered significant at values of *p*<0.05. In this study distribution of Ct values was compared among aborted bovine, ovine, caprine, buffaloes and camelid fetuses using ANOVA (Analysis of Variance) test.

Results

In this study, a total of 892 bovine, 810 ovine, 786 caprine, 604 buffaloes and 618 camelid abomasal content of aborted fetuses from different parts of Iran were tested for *Brucella* spp. using a conventional and real-time PCR assays. The presence of *Brucella* DNA detected by single PCR in abomasal contents of aborted fetuses samples were from 1124 out of 3710 animals (30.29%) (Fig 1). The incidence of *Brucella* spp. in bovine, ovine, caprine, buffaloes and camelid aborted fetuses was 31.5%, 27.65%, 27.86%, 32.94% and 32.52% in Iran (Table 1).

After real-time PCR, BMEII0466 and BruAb2-0168 gene were distinguished in 608, 492 and 90 out of 1124 specimens that were positive for *B. melitensis*, *B. abortus* and both bacteria, respectively (Fig 2). Results showed that 5.04% and 25.89%, 20.86% and 6.04%, 24.68% and 2.79%, 9.1% and 22.68% and finally 8.25% and 23.13% of aborted bovine, ovine, caprine, buffalo and camelid fetuses were diagnosed positive by real-time PCR for incidence of *B. melitensis* and *B. abortus*, respectively. On the other hand, by using of BMEII0466 and BruAb2-0168 gene specific primers, none of the two species (*B. melitensis* and *B. abortus*) were found in 29 samples (Table 1). Results shown in Table 1 indicated that the incidence of other species of *Brucella* in aborted buffalo fetuses was higher than the other species (1.15%). In this study, the incidence of both bacteria in aborted bovine, ovine, caprine, buffalo and camelid fetuses was 1.56%, 2.09%, 1.65%, 3.47% and 4.04%, respectively. According to Table 1, aborted camelid fetuses with 4.04% had the highest incidence of both bacteria.

Statistical analysis showed significant differences (*p*<0.01) between *B. abortus* and *B. melitensis* that were detected in abomasal contents of aborted bovine, ovine, caprine, buffaloes and camelid

fetuses and *p*<0.05 between presences of aborted *Brucella* spp. in bovine and caprine, buffalo and camel fetuses. In addition, CT values which were obtained from real-time PCR for presence of *B. melitensis* had significant differences (*p*<0.01) between aborted caprine and buffaloes, camelid and bovine fetuses while CT values which were obtained from real-time PCR for presence of *B. abortus* had significant differences (*p*<0.01) between bovine with ovine and caprine and *p*<0.05 between bovine with buffaloes and camelid aborted fetuses.

These results indicated that the incidence of *B. abortus* and *B. melitensis* in aborted bovine and caprine fetuses was higher than in the others, respectively. The prevalence of *B. abortus* in aborted camelid and buffaloes fetuses is higher than *B. melitensis*. Therefore, *B. abortus* is recognized as the main causes of abortion in buffaloes and camelid fetuses in Iran. The results indicated that buffalo and ovine had the highest and lowest infection of *Brucella* spp. in aborted fetuses. This study showed that the infections of these bacteria were high in aborted fetuses of Iran's animal herds.

Discussion

This present study shows that molecular methods such as conventional and real-time PCR are accurate, trustful and rapid assays for detection and segregation of *B. abortus* and *B. melitensis* in aborted bovine, ovine, caprine, buffaloes and camelid fetuses but the real-time PCR assay has some advantages compared to the conventional PCR; it is an important diagnostic tool yielding reliable and reproducible results, it does not require post-PCR analysis (gel electrophoresis, hybridization), and the risk of cross contamination is limited than conventional method, but the real-time PCR is more expensive than conventional PCR. Many studies have shown that the conventional method for detecting *Brucella* spp. is technically time-consuming and labor-intensive than real-time PCR assay (Bogdanovich et al., 2004; Yang et al., 2007).

Table 1 Distribution of *B. melitensis*, *B. abortus* and *Brucella* spp. in aborted bovine, ovine, caprine, buffalo and camelid fetuses by evaluation of conventional and Real-Time PCR assays in Iran.

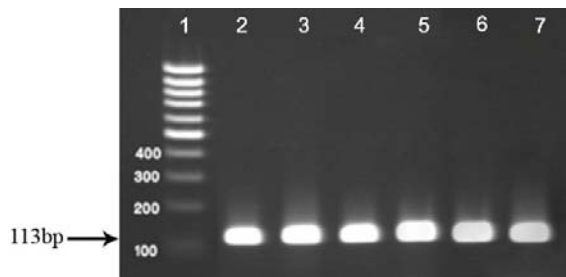| Species	Conventional PCR (%)	Real-Time PCR (%)			
		B. melitensis	*B. abortus*	Both bacteria	Unknown
Bovine	31.5 (281/892)	5.04 (45/892)	25.89 (231/892)	1.56 (14/892)	0.56 (5/892)
Ovine	27.65 (224/810)	20.86 (169/810)	6.04 (49/810)	2.09 (17/810)	0.74 (6/810)
Caprine	27.86 (219/786)	24.68 (194/786)	2.79 (22/786)	1.65 (13/786)	0.38 (3/786)
Buffalo	32.94 (199/604)	9.1 (55/604)	22.68 (137/604)	3.47 (21/604)	1.15 (7/604)
Camelid	32.52 (201/618)	8.25 (51/618)	23.13 (143/618)	4.04 (25/618)	1.13 (7/618)
Total	**30.29 (1124/3710)**	**16.38 (608/3710)**	**13.26 (492/3710)**	**2.42 (90/3710)**	**0.78 (29/3710)**

Figure 1 Conventional PCR for detection of *Brucella* spp. 1 is 100 bp ladder, 2-6 is positive samples for aborted bovine, ovine, caprine, buffalo and camel fetuses and 7 is positive control.
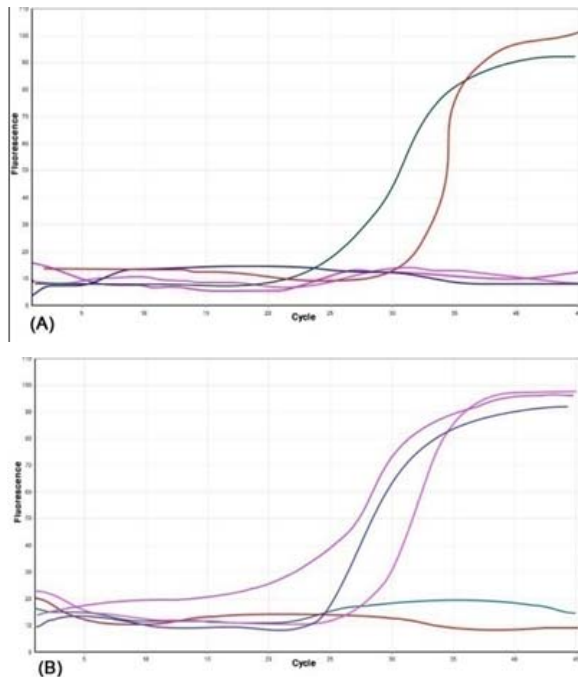

Figure 2 Real-time PCR for differentiation of *B. abortus* (A) and *B. melitensis* (B). CT less than 43 was considered as positive.

In addition to above, the real-time PCR assay, which was used in this study, allows precise detection of two *Brucella* spp. (*B. abortus* and *B. melitensis*) and can simplify the procedure by testing presumptive *Brucella* genome taken directly from abomasal contents of aborted fetuses. According to some features, the six different species of *Brucella* were recognized, all of these species show high degrees of genetic similarity. Therefore, conventional PCR technique, most often, is not able to precisely differentiate between *Brucella* species. Multiplex-PCR can be used for simultaneous detection and segregation of this two pathogens, but studies showed a high sensitivity and specificity of real-time PCR against multiplex PCR (Yu and Nielsen, 2010). In addition, multiplex PCR need post-PCR analysis like gel electrophoresis and this can make it tedious and time-consuming.

In this present study, the sensitivity and specificity of conventional and real-time PCR assays for detection of bacteria in aborted fetuses were 94%, 90% and 100%, 100%. Therefore, real-time PCR is more sensitive and specific than conventional PCR for detection of these two bacteria. The high incidence of

B. melitensis or *B. abortus* in abomasal content samples of unspecific hosts of this present study may show that these animals had been maintained in close association with infected sheep and cattles.

The prevalence of total *B. abortus* observed in bovine in this study (25.89%) is higher than Egypt (5.44%) (Samaha et al., 2008), Ethiopia (4.9%) (Mekonnen et al., 2010), Punjab (India) (20.67%) (Aulakh et al., 2008) and Sokoto State (25.25%) (Junaidu et al., 2011), but our results are lower than kenya (77.5%) (Namanda et al., 2009). The incidence of *B. melitensis* observed in aborted caprine fetuses of this study (24.68%) is lower than Turkey (31%) (Leyla et al., 2003) and Jordan (27%) (Al-Majali, 2004). A previous study from Iran showed that the incidence of *Brucella* spp. in aborted bovine, ovine and caprine fetuses were 22.8%, 6.3% and 3.8%, respectively (Moshkelani et al., 2011). In Turkey, the antibodies against *B. abortus* were detected in aborted dairy cattle as 68.1% by the Competitive Enzyme- Linked Immunosorbent Assay (C-ELISA) (Gen et al., 2005). The incidence of *B. abortus* observed in Buffalo in this present study (22.68%) is higher than Egypt (0.3%) (Refai, 2002), Punjab (India) (13.4%) (Dhand et al., 2005), but are lower than Africa (30%) (Waghela and Karstad, 1986). The prevalence of Brucellosis in the Camels of this present study (23.13%) is higher than Pakistan (2.47%) (Ajmal et al., 1989), Libia (3.76%) (Faraj et al., 1991), Somalia (1.9%) (Baumann and Zessin, 1992) and Sudan (6.95%) (Yagoub et al., 1989).

Studies showed that the higher prevalence of brucellosis (23.8%) may occur in camels which are kept together with ruminant species (Musa et al., 2008). Seroprevalence of camel brucellosis appear to follow two distinct patterns, a prevalence below 5% in nomadic or extensively kept camels and a high prevalence around 8-15% in camel kept intensively or semi intensively (Abbas and Agab, 2002). The results of our study showed that both *B. abortus* and *B. melitensis* could infect camels, but the incidence of *B. abortus* was higher than *B. melitensis* and this finding was similar to previous studies (Agab et al., 1994).

Studies indicated that camels were not the primary hosts of *Brucella*, but they could be infected with both *B. abortus* and *B. melitensis* (Cooper, 1991) and consequently, the prevalence was dependent on infection rate of primary hosts being in contact with them. To the author's knowledge, spread of brucellosis in camels depends on the *Brucella* species prevalent in other animals sharing their habitat and on the husbandry methods of the different species. Studies from various parts of Iran (Salari, 2002; Rafeipour et al., 2007; Nowroozi-Asl et al., 2007) indicated that Brucellosis was one of the most important endemic disease in Iran.

To the author's knowledge and the previous studies, the detection of *Brucella* spp. by PCR in aborted clinical samples was only evaluated in cattle and sheep (Fekete et al., 1992; Çetinkaya et al., 1999) and there has been no report on the incidence of *Brucella* spp. in aborted camel, goat and buffalo fetuses by PCR method. Seroprevalence of brucellosis in aborted bovine, ovine and camel fetuses showed

that *Brucella* infections contributed significantly to abortion in cattle (odds ratio (OR), = 4.7; 95%CI, 2.0, 10.8) and goats (OR = 6.9; 95%CI, 2.2, 21.7), but not in camels (Megersa et al., 2011). Studies indicated that the multivariable logistic regression model on both individual and herd levels revealed large herds in contact with small ruminants are as risk factors for prevalence of brucellosis (Al-Majali et al., 2008).

In extensive management system the prevalence of brucellosis among various species of animal is low (Mohammed et al., 2011). Camels and buffaloes are not known to be primary host for any of *Brucella* organisms but many studies showed that they were susceptible to both *B. abortus* and *B. melitensis* (Musa and Shigidi, 2001; Teshome et al., 2003).

Previous studies showed that Saudi Arabia, Iran, Syria, Jordan and Oman had the highest incidence of brucellosis among the countries of the Near East region (Refai, 2002). The results of this present study showed that the buffalo and camelid species could be the important reservoir for transmission of this zoonosis to human in Iran. There are many reports on *B. abortus* abortion in camels, but infection of camels with *B.melitensis* is rare (Zowghi and Ebadi, 1988).

This study showed that *B. abortus* is the main cause of brucellosis in buffaloes (22.68%) and camelid (23.13%), while the presence of *B. melitensis* in Buffaloes and camelid was 9.1% and 8.25% respectively. In total, brucellosis causes great economic losses in Iran. Previous report from Iran (East of Iran) indicated that the prevalence rate of brucellosis during 2002-2006 in human was 37/100,000, in sheep and goat was 340/10,000 and in cattle was 56/10,000 (Bokaie et al., 2008). Despite the advances made in surveillance and control, the prevalence of brucellosis is increasing in many developing countries due to various sanitary, socioeconomic, and political factors (Pappas et al., 2006). There are many factors that may effect the incidence and epidemiology of brucellosis such as processing milk and milk products, socioeconomic status, climatic conditions, social customs, food habits, husbandry practices and environment hygiene (Mantur et al., 2007).

To our knowledge, this study is the first report of direct detection and segregation of *B. melitensis* or *B. abortus* by application of conventional and real-time PCR assays in aborted bovine, ovine, caprine, buffaloes and camelid fetuses in Iran. In conclusion, Brucellosis is one of the most important endemic zoonotic diseases in Iran. Our results indicated that in Iran, animal brucellosis affects almost all domestic animals such as bovine, ovine, caprine, buffalo and even camel. We hope that the real-time PCR method, which was introduced in this study as an accurate, safe, fast, sensitive and specific assay for detection and segregation of *B. melitensis* and *B. abortus* in clinical samples, will continuously be used.

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