Original Article

Detection of *Brucella* spp. in the Sheep and Goats Milks from Southeastern Iran Using Culture and PCR

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Introduction: Brucellosis is a zoonotic disease caused by the members of the genus *Brucella*. This bacterium is transmitted to humans through exposure to infected animals or via consumption of contaminated dairy products. Cultivation of the bacteria or amplification of its DNA using polymerase chain reaction (PCR) are conventional diagnostic approaches for definitive identification of *Brucella* in raw milk. **Method:** We collected 530 milk samples from 485 healthy animals, and 45 animals with a history of abortion from Kerman province, southeast Iran. The specimens were first cultured in Eugon broth and then were subcultured on *Brucella* agar. Gram smears from colonies for characterization of the bacteria were prepared. Also, DNA extraction and PCR amplification of IS711 fragment were performed to detect *Brucella* DNA in the milk samples. **Results:** The culture method detected *Brucella* Spp. in 10 milk samples including two samples from apparently healthy animals (1 sheep sample, and 1 goat sample) as well as eight samples from animals with abortion history (6 sheep samples, and 2 goat samples). PCR identified *Brucella* DNA in 43 samples including those from healthy sheep (n=4) and goats (n=9), as well as animals milk with abortion history (7 sheep, and 23 goats). The proportion of positive samples detected by PCR method was significantly higher than culture method (*P*=0.014). **Conclusion:** The PCR assay turned to be a convenient method for detection of *Brucella* contamination of raw milk and can be used as a reliable tool for surveillance and screening of contaminated milk. *J Med Microbiol Infect Dis, 2017, 5 (3-4): 40-42. DOI: 10.29252/JoMMID.5.3.4.40*

Keywords: *Brucella*, PCR, Culture, Goat, Sheep.

INTRODUCTION

Brucellosis is an emerging zoonotic bacterial disease with a significant impact on both animal and human health worldwide. In cattle, it causes abortion, infertility and decreases milk yield [1] and in humans, can cause a chronic infection reflecting occupational exposure or consumption of the contaminated dairy products. Various techniques are used to detect *Brucella* contamination of raw milk including isolation of bacteria by culture, amplification of *Brucella* DNA by PCR, and indirect assays. Cultivation is the gold standard method for diagnosis of brucellosis; however, due to low sensitivity, it is often unsuccessful. It is also time-consuming and hazardous for laboratory personnel [2]. In contrast to bacterial culture method, molecular assays are more sensitive. PCR assays, as sensitive, specific and relatively inexpensive tools have been used for detection and characterization of *Brucella* at genus, species and biovar level in abortion samples [3, 4]. This study aimed to detect *Brucella* bacteria in raw milk by culture method and a PCR assay targeting a specific region of the *Brucella* genome, the IS711 fragment. The sensitivity of both methods was also compared.

MATERIAL AND METHODS

Milk samples. From April 2015 to Jun 2016, 530 milk samples from both healthy and infected domestic animals (80 herds) were collected from different regions of Kerman, southeast of Iran (Fig. 1). The samples were from 45 infected animals with a history of abortion (32 goats and 13 sheep) and 485 apparently healthy animals (290 goats and 195 sheep) based on clinical manifestations.

Bacteria Culture. The milk samples were centrifuged at 3500×g for 20 min, and the sediment and the cream layer was cultured in Eugon broth followed by incubation at 10% CO_2 for 4 days at 37°C. Amounts of 100 µl from Eugon broths were subcultured in *Brucella* agar containing inactivated horse serum and the antibiotics, polymyxin Bsulfate, Bacitracin, Nystatin, Cyclohexamide, Nalidixic acid and Vancomycin, and incubated for 5 days at 37°C with 10% CO_2. The resulting colonies were identified by Gram staining.

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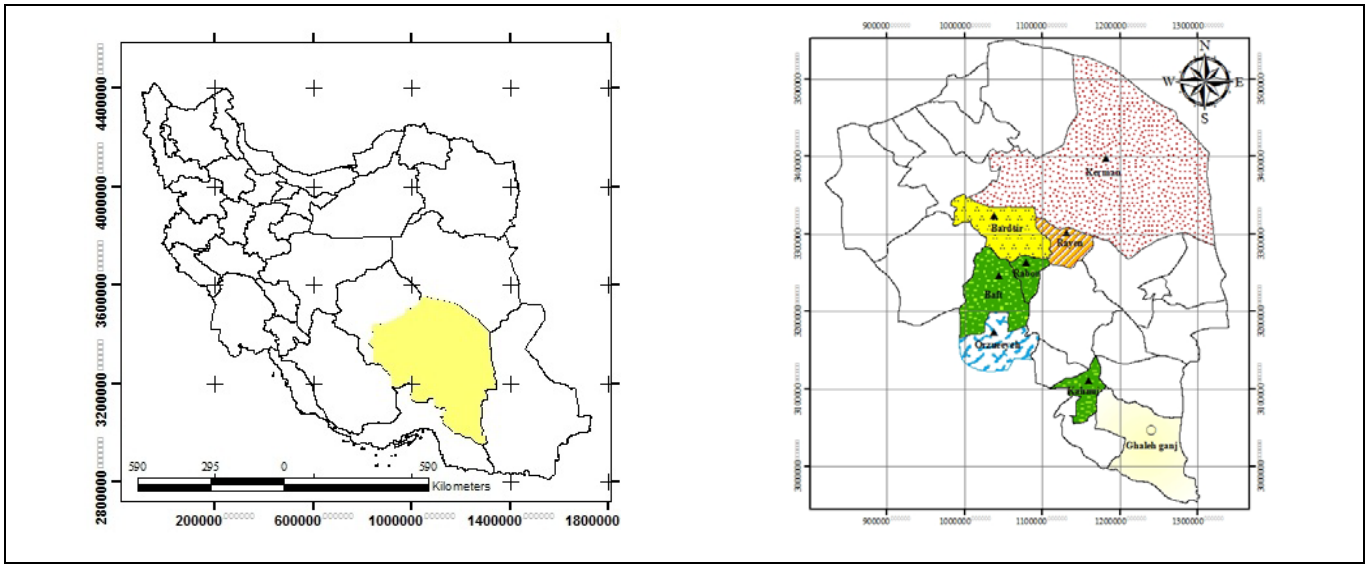

Fig.1. Geographic distribution of sampling in this study

PCR amplification of samples. In this study, rapid isolation of mammalian DNA method was applied for the *Brucella* DNA extraction [5], and the DNA samples were stored at -20°C until used. PCR amplification was performed to evaluate the presence of *Brucella* DNA in milk samples by targeting the 317 bp IS711 fragment using the primers 5'-GAGAATAAAGCCAACACCCG-3' and 5'-GATGGACGAAACCCACGAAT-3' designed by others [6]. The 25 µl PCR mixtures contained 2.5 U of Easy-A *Taq* DNA polymerase, 1× Easy-A *Taq* buffer, 2 mM $MgCl_2$, 2.5 mM dNTPs, 40 pmol of each primer, and 100 ng of purified genomic DNA. Polymerase chain reaction was performed as follows: 95°C for 3 min for denaturation, 30 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s, and finally 72°C for 10 min [6].

Statistical analysis. Statistical analysis was done using SAS 9.1 software. We used McNemar's test for comparing the two diagnostic methods as both diagnostic experiments were carried out on the same samples (P <0.05).

RESULTS

Culture. Among all samples examined, 10 (1.88%) were found positive by culture and Gram staining tests. Among these positive samples, two samples were from apparently healthy animals (1 sheep and 1 goat), and 8 were from animals with a history of abortion (2 sheep and 6 goats) (Table 1).

PCR. PCR amplification of IS711 fragment yielded a 317 bp band in 43 (8.11%) milk samples, 11 and 32 samples from the sheep and goats, respectively (Fig. 2). Among these positive samples, 13 belonged to apparently healthy animals (4 sheep and 4 goats), and 30 to animals with a history of abortion (7 sheep and 23 goats) (Table 1). The proportion of positive samples detected by PCR method was significantly higher than the culture method (P=0.014).

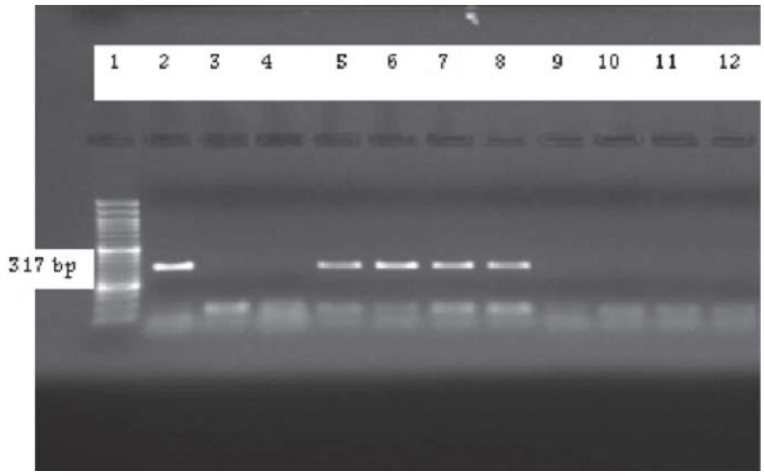

Fig. 2. Detection of *Brucella* DNA in milk collected from southern Iran by IS711 PCR. Lane 1, molecular size markers. (50 bp DNA ladder); lane 2, positive control; lane 3, NTC (negative template control); lane 4, extraction control; lanes 5 to 8, positive samples; lanes 9-12, negative samples.

Table 1. Number of goats and sheep milk samples used in this study

Number of samples (apparently healthy)		PCR$^+$ in samples (apparently healthy)		Culture$^+$ in samples (apparently healthy)		Number of samples (history of abortion)		PCR$^+$ in samples (history of abortion)		Culture$^+$ in samples (history of abortion)	
Goats	**Sheep**	**Goats**	**Sheep**	**Goat**	**Sheep**	**Goats**	**Sheep**	**Goats**	**Sheep**	**Goats**	**Sheep**
290	195	9	4	1	1	32	13	23	7	6	2
485		13		2		45		30		8	

DISCUSSION

The primary purpose of the present study was to compare the sensitivity of PCR and culture methods for *Brucella* detection in milk samples from 530 animals (322 goats and 208 sheep). The number of positive *Brucella* samples were significantly higher with PCR (8.11%) compared with the culture method (1.88%). All the 10 culture-positive milk samples were also positive by PCR confirming the higher sensitivity of this assay compared to the culture method. In a study by Hamdy and Amin (2002), out of 103 milk samples from different domestic animals (52 cows, 21 ewes, 18 goats, 12 camels), PCR identified *Brucella* DNA in 53 samples while the bacteria growth was observed in 46 cultures [7]. In another study in Turkey, on 102 sheep milk samples, the PCR method detected *Brucella* DNA in 24 samples (23.5%) while only 8 (7.8%) were positive by culture methods [8]. Our results confirmed that culture was not of high sensitivity for identification of bacteria in milk sample as fastidious bacteria such as *Brucella* are hard to grow. The negative PCR results of milk samples from 15 animals with a history of abortion does not necessarily reflect the lack of infection in animals as the bacteria might have lodged themselves in the lymph nodes and not contaminated the milk during sampling or a small undetectable number of bacteria might be present in the samples [3]. The detection of *Brucella* spp. by both PCR and culture in healthy animals (Table 1) suggests that infected animals sometimes might be asymptomatic with the bacteria being present in reproductive organs with no adverse sign in the animals [9].

Brucellosis is one of the critical bacterial diseases in Iran. The milk PCR method can be applied as a conventional screening method to reduce outbreaks of brucellosis in livestock. Our results confirmed that the PCR assay has several advantages over culture method such as more sensitivity and being safe and cost-effective.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

ETHICAL APPROVAL

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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