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Detection of *Brucella melitensis* from Aborted Caprine Fetuses in Iran

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Abstract: *Brucella melitensis* is a zoonotic pathogen that caused abortion and Malta fever in small ruminants and human, respectively. This study was conducted to determine the prevalence rate of *B. melitensis* in aborted fetus samples from caprine herds in Chaharmahal va Bakhtiari province, Iran., 140 aborted fetus samples from 17 Lori-Bakhtiari goat herds were tested. DNA was obtained directly from the abomasal contents of aborted fetuses and PCR was performed by primers derived from the omp31 gene sequence of the *B. melitensis*. Out of all samples 16.4% gave positive by this molecular methods. The results showed that PCR assay may be applied to detect *B. melitensis* directly from abomasal contents of goat and therefore could be a valuable diagnostic or screening test for herds with Brucellosis.

Key words: Caprine · Fetuses · Lori-Bakhtiari goat · *Brucella melitensis* · PCR

INTRODUCTION

Brucella melitensis is an economically important infectious disease of small ruminants worldwide, characterized by abortion and infertility [1]. Also, this bacterium occurs occasionally in some cattle herds. Pregnant cows may abort and shed *B. melitensis* in their milk [2]. Infected animals shed highly stable bacteria in urine, faces, milk and through placental and birth fluids. Small ruminants get infectious after either abortion or full-term parturition. Goats usually shed *B. melitensis* in vaginal discharges for at least 2 to 3 months, but shedding usually ends within three weeks in sheep [3, 4].

Brucella has been rated by the WHO as one of the most important zoonosis, as it is very pathogenic to humans, causing the disease known as Malta fever. The clinical disease caused by *B. melitensis* is more severe compared to that caused by *B. abortus* and *B. suis* in humans [5]. The prevalence of the disease in humans is mainly dependent on the animal reservoir, especially high rates of brucellosis in sheep and goats and socioeconomic situation of the countries [6].

There are various methods for diagnosis of brucellosis such as culture, serological and molecular methods. Culture methods are well established for brucellosis but highly dangerous to laboratory workers, difficult and lengthy process that requires experienced technicians [7]. Serologic methods are not conclusive, because not all infected animals produce detectable levels of antibodies and because cross-reactions with antigens other than those from *Brucella* can give false-positive results [8]. Therefore, molecular techniques like PCR has the potential to meet the need for better diagnostic tools for several infectious diseases caused by fastidious or slow growing bacteria [9].

The objective of the present study was to determine the prevalence rate of *B. melitensis* in aborted fetus samples from Lori-Bakhtiari goat of Chaharmahal va Bakhtiari provinces, Iran using a PCR assay.

MATERIALS AND METHODS

Sample and DNA Extraction: From March to May 2010 a total of 140 caprine aborted fetuses were collected from 17 Lori-Bakhtiari goat farms of Chaharmahal va Bakhtiari provinces, Iran. All clinical samples sent under refrigeration to the Biotechnology Research Centre of Islamic Azad University of Shahrekord. DNA was extracted from stomach contents of aborted fetuses by using DNA extraction and purification kit (Cinnagen, Tehran, Iran) according to manufacturer's instruction.

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Primer: Detection of *Brucella melitensis* was performed by amplification with the following primers: 5'-TGACAGACTTTTTCGCCGAA-3' (forward) and 5'-TATGGATTGCAGCACCGC-3' (reverse), derived from the published DNA sequence of 28–31 kDa outer membrane protein (omp31) of *B. melitensis* resulting in a 720 bp product [10].

Analysis of Samples with Single PCR: The PCR assay was performed in a final volume of 25 µL mixture containing 50 mmol KCl, 10 mmol Tris-HCl (pH 8.3), 1.5 mmol $MgCl_2$, 0.2 mmol of each deoxynucleotide triphosphate, 0.5 µmol of each primer, 1.25 unit Taq polymerase (Cinnagen, Tehran, Iran) and 5µL of DNA template.

Reactions were initiated at 94°C for 5 min, followed by 32 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 2 min and a final elongation step at 72°C for 10 min, with a final hold at 4°C. Amplified samples were analyzed by electrophoresis (120 V/208 mA) in 1.5% agarose gel and stained by ethidium bromide. A molecular weight marker with 100 bp increments (100bp ladder fermentas) was used as size standard.

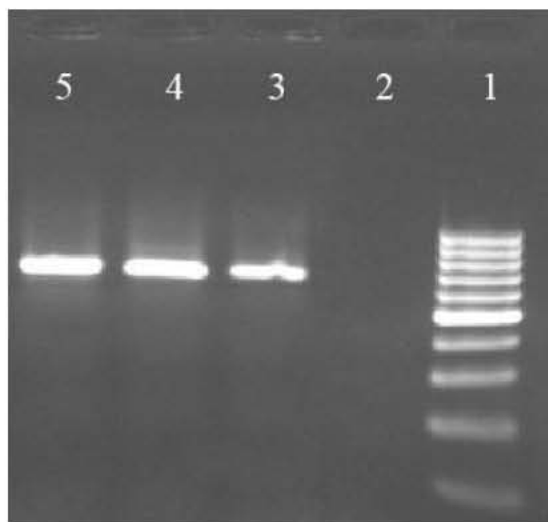

Fig. 1: PCR assay for the detection of *Brucella melitensis* from aborted Caprine fetuses in Iran. Lanes 1: 100bp DNA ladder; 2: negative control; 3 and 4: positive samples from aborted fetuses; 5: positive control

Table 1: Distribution of *Brucella melitensis* aborted fetus-positive samples from three goat herds during spring, 2010 in Chaharmahal va Bakhtiari province, Iran

Goat herd No.	March	April	May	Total of positive samples
2	-	+, +, +	+	4
10	+, +	+, +, +	+, +	7
15	+, +, +	+, +	-	5

RESULTS AND DISCUSSION

To best our knowledge, the present study is the first report of prevalence of *Brucella melitensis* from aborted Caprine fetuses in Iran using PCR.

PCR amplification of the *B. melitensis* specific outer membrane protein (omp31) gene agarose gel analysis of the amplified products showed a single band of 720 bp for each of the positive aborted fetus samples (Fig. 1). In total, 23 of 140 (16.4%) aborted caprine fetus samples were positive. The positive samples were from 10 of 17 (58.8%) Lori-Bakhtiari goat herds in Chaharmahal va Bakhtiari provinces, Iran. Sixteen of 23 positive aborted fetus samples were from three goat herds (herd number 2, 10 and 15) with 4, 7 and 5 positive samples, respectively (Table 1).

This result is approximately similar to a recent report in Jordan that showed a prevalence of *B. melitensis* of 17.5% (33/188) in aborted Jordanian goat samples using PCR-RFLP method [11]. In another study conducted in Turkey, 10% of goat and 38.1% of sheep aborted fetus sample were given positive for *B. melitensis* [12]. Detection of *Brucella melitensis* DNA in the milk of sheep after abortion by PCR assay was conducted in Turkey. In their report, a total number of 8 (7.8%) *Brucella* positive samples were detected only in Farrell's modified serum dextrose agar of the 102 milk samples. All Brucella organisms were identified and biotyped as *B. melitensis* biovar 3 [8]. In another study of Turkey, aborted sheep fetuses were collected during the lambing seasons of 2004 to 2006. *B. melitensis* was isolated from 25 (29.76%) of 84 lungs and stomach contents [13]. Also, Teixeira-Gomes et al., [14] and Leyla et al., [15] isolated *Brucella melitensis* from vaginal swabs and aborted fetuses in sheep and goats.

In conclusion, with all the precautions in this infection disease throughout the world it is not only still fully eradicated but also, it has a high prevalence in some areas like Iran. Control of brucellosis requires elimination of infected animal and vaccination of healthy ones in order to reduce the risk for those in regular contacts.

In summary, the results of this study indicate that *Brucella melitensis* was detected as a causative agent of abortion in the Lori bakhtiyari goat in Iran. In addition, PCR assay could become a valuable diagnostic or screening test for goat herds with Brucellosis.

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