

## Simultaneous Detection of *Brucella* sp. and *Salmonella abortus ovis* by Multiplex PCR

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**Abstract:** *Brucella* sp. and *Salmonella abortus ovis* are important causes of ovine abortion around the world. Both Bacteria can be serologically diagnosed, but many factors may cause false positive and negative results. Direct methods based on bacteriological isolation are usual, but they are difficult, time consuming and dangerous. Polymerase Chain Reaction (PCR) have been successfully geted usefull details and discribing the detection of *Brucella* sp. and *Salmonella abortus ovis*. The detection of these agents in aborted ovine fetuses by multiplex PCR is described. The mPCR was applied to 54 fetal stomach contents. 10 samples collected from ovine fetus were *Brucella* sp. 24 samples collected were *Salmonella abortus ovis*. Fourteen samples collected were negative and 6 samples collected were *Brucella* sp. and *Salmonella abortus ovis*. Simplicity and the possibility of detection of both bacteria in a single tube reaction support the use of the mPCR is the commen method for microbiological diagnosis.

**Key words:** Multiplex PCR, *Brucella* sp., *Salmonella abortus ovis*, ovine fetus

### INTRODUCTION

*Brucella* sp. and *Salmonella abortus ovis* are widly distributed around the world. Reproductive such as abortions and premature births may be the only clinical signals of these bacterial diseases in pregnant ewes. Both diseases can be diagnosed by detection of serum specific antibodies but these methods are presumptive because many factors may cause false positive and negative results.

Direct methods based on the demonstration of the bacteria in the host are the most objective diagnostic procedures. Bacteriological isolation is usually employed, but it is difficult, time consuming and dangerous (Kirkbride *et al.*, 1990; Leyla *et al.*, 2003; Nielsen and Duncan, 1990; Salehi *et al.*, 2006).

After the development of the Polymerase Chain Reaction (PCR), some papers described it is use for the diagnosis of *Brucella* sp. (Herman and Ridder, 1992; Romero *et al.*, 1995) and *Salmonella abortus ovis* (Beuzon *et al.*, 1997; Masala *et al.*, 2007). Multiplex PCR (mPCR) is a PCR derived procedure where multiple target DNA sequences can be detected in a single reaction (Richtzenhain *et al.*, 2002).

This paper describes a mPCR by novel primers for the detection of both *Brucella* sp. and *Salmonella abortus ovis* DNA in an aborted ovine fetuses.

### MATERIALS AND METHODS

**Reference bacterial strains:** *Brucella abortus* strain 119-3 and *Salmonella abortus ovis* were kindly supplied by Dr. Tadjbakhsh Hassan of the laboratory of bacterial of the faculty of veterinary medicine of the University of Tehran, Iran.

**Ovine clinical samples:** Clinical samples from 54 aborted ovine fetus sent under refrigeration to the Biotechnology Research Center of Islamic Azad University of Shahrekord for bacteriological examination were studied. All of the samples had only abomasal contents. Whole abomasal contents were stored at -20°C until required for DNA extraction.

#### mPCR

**Extraction protocol:** Genomic DNA directly isolated from abomasal contents by Cinnagen DNA™ kit (IRAN).

**DNA amplification:** PCR assays for the detection of *Brucella* sp. (PCR/Bruce) and *Salmonella abortus ovis* (PCR/SAO) was done. The expected size of amplicons 243 bp for *Brucella* sp. and 172 bp for *Salmonella abortus ovis*, the mPCR assay employed the novel primers of PCR assays.

Bruce: 5'-CTATTA TCC GAT TGG TGG TCT G-3' and  
Bruce: 5' -GGT AAA GCG TCG CCA GAA GG-3' for  
*Brucella* sp. and  
SAO: 5'-GCC GAA GAT GAG TGT GTC CAG TT-3' and  
SAO: 5' -CCG TGT TCT TAC CCA CCG TAT- 3' for  
*Salmonella abortus ovis*. The mPCR assay was carried  
out in 0.5 mL microtubes under following conditions  
initial denaturation at 97°C for 4 min by 30 cycles of  
denaturation at 94°C for 1 min, annealing at 57°C for  
40 sec extension at 72°C for 40 sec and final extension at  
72°C for 3 min.

**Visualization of PCR products:** The PCR products were  
visualized after electrophoresis in 2% agarose gels and  
stained by ethidium bromide (Sambrook *et al.*, 2001). A  
molecular weight marker with 100 bp increments  
(100 bp ladder fermentas) was used as size standards.

## RESULTS

DNA was extracted successfully from all of the  
samples and evaluation of DNA efficacy on agarose gel  
showed that the result were desirable.

After PCR amplification this result were obtained: In  
54 fetal stomach contents, 10 samples are *Brucella*  
possetive and in 24 of them *Salmonella* were detected  
and 6 sampels contain both *Brucella* and *Salmonella* and  
14 samples collected were negative.

## DISCUSSION

*Brucella* sp. and *Salmonella abortus ovis* were find  
widly in Iran. Brucellosis and salmonellosis are imprtant  
economic disease in livestock enterprise as it induces  
abortion in infected animals (Fig 1).

The disesae very often spreads from animal to animal  
in a herd by several modes of transfer, chief among these  
being contact with infected discharges from an aborted  
ewe and it is fetus achievement of an infallible diagnosis  
is a tedious process, since isolation is influenced by a  
number of factors, such as highly fastidious growth  
requirements, a lesser number of viable organism in the  
sample, delay in transportation (leading to putrefaction),  
earlier treatment with chemotherapeutics. Also, a  
prolonged incubation period for isolation may lead to  
failure in its isolation. The PCR technique has increasingly  
been used as a supplementary method in a *Brucella*  
diagnosis and *Salmonella abortus ovis* detection by  
simultaneously amplifying more than one locus in the  
same reaction, mPCR has been identified as a rapid and  
convenient screening assay, with both clinical and  
research applications. Simultaneous detection of two

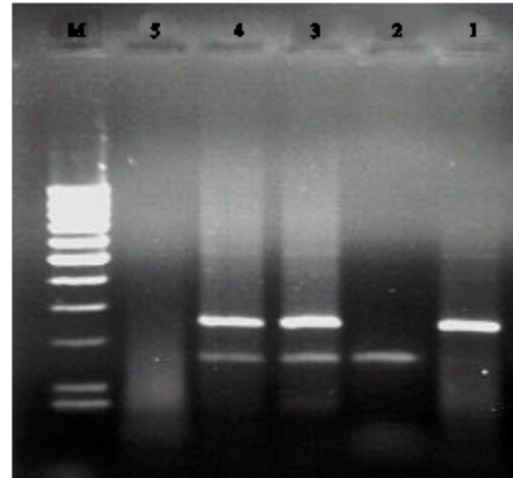


Fig. 1: Multiplex PCR for the Detection of *Brucella* sp. and *Salmonella abortus ovis*. Lane 1 to 3, different samples from aborted ovine fetus and lane 4 to 5 are positive and negative control, respectively

major potential pathogenic bacteria in fetal stomach  
contents has been demonstrated in the present study by  
analyzing a single sample using mPCR. The results show  
that developed mPCR assay was able to successfully  
detect *Brucella* sp. and *Salmonella abortus ovis*.

The following reasons could be listed to recommend  
the use of the mPCR proposed in this study for routine  
diagnosis of *Brucella* sp. and *Salmonella abortus ovis*  
in ovine abortions. The simplicity and speed of the  
procedure. The possibility of detection of both  
*Brucella* sp. and *Salmonella abortus ovis* in a single  
tube reaction.

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