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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 bactera 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.

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Bruce: 5′ -CTA TTA 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 deneturation 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 contein 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

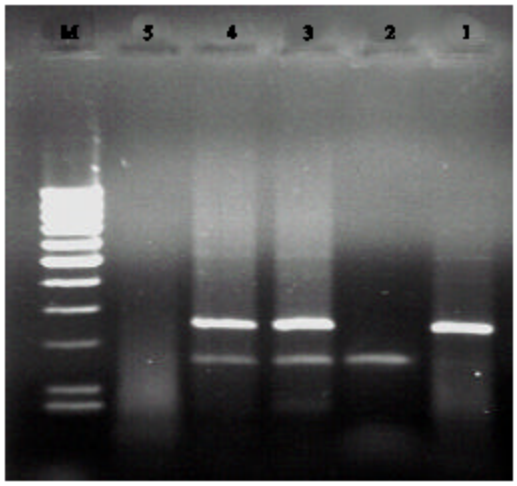

Fig. 1: Multiplex PCR for the Detection of *Brucella* sp. and *Salmonella abortus ovis*. Lane 1 to 3, different samples from aborted ovine fetus and lane 4 to 5 are positive and negative control, respectively

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 possibitity of detection of both *Brucella* sp. and *Salmonella abortus ovis* in a single tube reaction.

REFERENCES

Beuzón, C.R., A. Schiaffino, G. Leori, P. Cappuccinelli, S. Rubino and J. Casadesús, 1997. Identification of *Salmonella abortusovis* by PCR amplification of a serovar-specific IS200 element. Applied Environ. Microbiol., 63: 2082-2085.

Herman, L. and H. De Ridder, 1992. Identification of *Brucella* sp. by using the polymerase chain reaction. Applied Environ Microbiol., 58: 2099-2101.

Kirkbride, C.A., 1990. Laboratory diagnosis of livestock abortion. Iowa State University Press, Ames, IA, pp: 260.

Leyla, G., G. Kadri and O. Umran, 2003. Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheep brucellosis using aborted fetus samples. Vet. Microbiol., 93: 53-61.

Masala, G., R. Porcu, C. Daga, S. Denti, G. Canu, C. Patta, and S. Tola, 2004. Detection of pathogens in ovine and caprine abortion samples from sardinia, italy, by PCR. J. Vet. Diagn. Invest., 19: 96-98.

Nielsen, K. and J.R. Duncan, 1990. Animal Brucellosis. CRC Press Inc., Boca Raton, Florida, pp: 450-455.

Richtzenhain, L.J., A. Cortez, M.B. Heinemann, R.M. Soares, S.M. Sakamoto, S.A. Vasconcellos, Z.M. Higa, E. Scarcelli and M.E. Genovez, 2002. Multiplex PCR for the detection of Brucella sp. and *leptospira*. DNA from aborted ovine fetuses. Vet. Microbiol., 87: 139-147.

Romero, C., M. Ggamzo and L. Lopez-Goni, 1995. Specific detection of *Brucella* DNA by PCR. J. Clin. Microbiol., pp: 615-617.

Salehi, M., E. Pishva, R. Salehi and M. Rahmani, 2006. Solation of Brucella abortus using PCR- RFLP analysis. Iranian J. Pub. Hlth., 35: 22-27.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. (3rd Edn.), Cold Spring Harbor, N.Y, Cold Spring Harbor Laboratory Press, Vol. 1,2.