



Molecular characterization of *Brucella abortus* from cattle and buffaloes

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Bovine brucellosis, a contagious disease found worldwide particularly in developing countries (OIE 2000), is caused by *Brucella abortus*, and is characterized by abortion in females and orchitis in males along with infertility in both sexes. Though isolation of the causative organism is the best method for confirmation of brucellosis but it is time consuming. PCR has been advocated as a reliable alternative of culture and isolation since it is a very sensitive technique that can directly detect DNA of the disease causing agent thereby could be used for establishing current infectious status. This assay is not only sensitive and fast but is also safe as per as human handling in lab is concern. Therefore, PCR based assay was used successfully for the diagnosis of various infectious diseases including brucellosis (Queipo-Ortuno *et al.* 1997).

In the present study 84 samples of vaginal mucus, placenta, cotyledons and foetal stomach contents from aborted cattle and buffaloes were inoculated on selective

media for the isolation. The selective media used was Brain Heart Infusion (BHI) agar with 7–10% defibrinated sheep blood along with 100mg/L of cyclohexamide, 6,000 unit/L of polymyxin B, 10mg/L of vancomycin and 5mg/L of trimethoprim. After inoculation for 3–5 days suspected colonies were checked for their morphological, cultural and biochemical properties (Quinn *et al.* 1994). To further ascertain *Brucella* agglutination test was done using standard antiserum (IVRI).

DNA was extracted from the broth culture of the above isolated bacteria indicated as per Wilson (1987). The purity and concentration of the extracted DNA was determined by spectrophotometer. DNA was directly extracted from different samples by standard phenol-chloroform method of DNA extraction (Sambrook and Russel 2001). PCR was standardized using F4 and R2 primers and B4 and B5 primers (Table 1).

For primers F4/R2 and B4/B5 amplification reaction

Table 1. Description of primers used in diagnostic PCR

Primer name	Primer sequence	Target gene	Size of amplicon/PCR	Reference frame
F4	5'- TCG AGC GCC CGC AAG GGG - 3'	<i>16S rRNA</i>	905bp	Romero <i>et al.</i> (1995)
R2	5'- AAC CAT AGT GTC TCC ACT AA - 3'			
B4	5'- TGG CTC GGT TGC CAA TAT CAA - 3'	<i>bcs31</i>	223bp	Baily <i>et al.</i> (1992)
B5	5'- CGC GCT TGC CTT TCA GGT CTG - 3'			

Table 2. *Brucella* PCR program

Stage	Step	Temperature °C (for F4/R2) primers	Duration (for F4/R2) primers	Temperature °C (for B4/B5) primers	Duration (for B4/B5) primers	No. of cycles
I	Initial denaturation	95	4 min	94	5 min	1
II	Denaturation	95	60s	94	60s	35
	Annealing	56	60s	65	60s	
	Extension	72	60s	72	60s	
III	Final extension	72	3 min	72	10 min	1

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mixture was prepared in a volume of 25 µl containing 1X PCR buffer, 1.5 mM MgCl₂, 200 µM each deoxynucleoside triphosphate, 20 pmol each primer, 1 U of *Taq* DNA polymerase and 3 µl of template DNA (~100ng). The PCR conditions used are given in Table 2.

PCR reactions were carried out in thermal cycler. In each PCR run, a positive control (*Brucella abortus* vaccine strain S-19) and a negative control (non template control containing PCR grade water) were included to monitor adequate performance of run and absence of cross contamination. Amplifications were detected and photographed after electrophoresis in a 1% agarose gel in the presence of ethidium bromide (1µg/ml) in gel documentation system.

In the present study *B. abortus* was isolated from 9 out of 84 (10.71%) different samples of vaginal mucous (35), foetal stomach contents (31), uterine discharges (7) and foetal membranes (11) from cattle and buffaloes. All the 9 isolates were confirmed by PCR using 2 sets of primers F4/R2 and B4/B5 which amplified 905bp and 223bp, respectively (Figs 1, 2). Twelve samples were found positive by PCR (Fig. 3) utilizing genus specific primers F4 and R2 targeting *16S rRNA* gene of *B. abortus* and amplified 905bp region. There are higher number of commensal bacteria and low number of *Brucella* in foetal membranes and vaginal mucus samples, which made isolation difficult. Fekete *et al.* (1990) also determined sensitivity of PCR with different concentrations of genomic DNA of *Brucella abortus* S-19.

Diagnostic potential of 3 conserved genes was tested and *bcs31* (*omp31*) was evaluated to be a good candidate for the diagnosis of *Brucella* infection to genus level (Bounaadja *et al.* 2009). The sensitivity of PCR was found

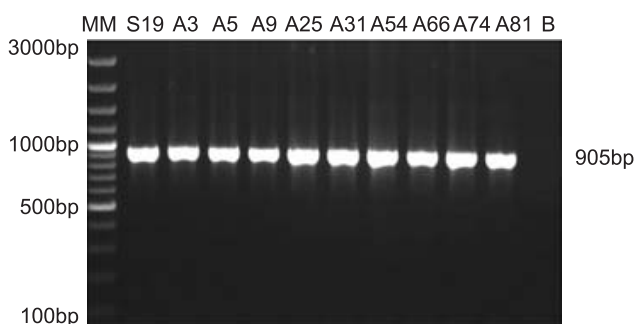


Fig. 1. Detection of *B. abortus* isolates using F4/R2 primers. Lane MM: molecular weight marker, Lane 1: positive control (S19 strain), lane A3 to A81: isolates, lane B: blank.

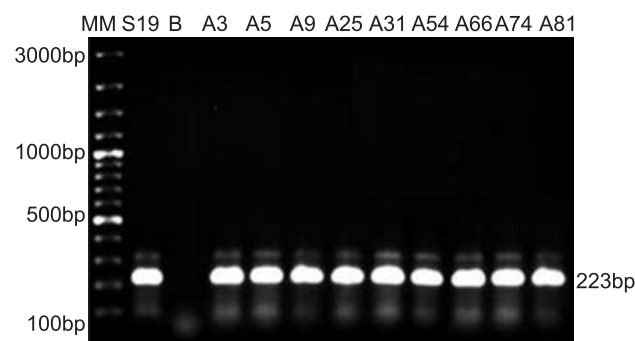


Fig. 2. Detection of *B. abortus* isolates using B4/B5 primers. Lane MM: molecular weight marker, lane 1: positive control (S19 strain), lane B: blank, lane A3 to A81.

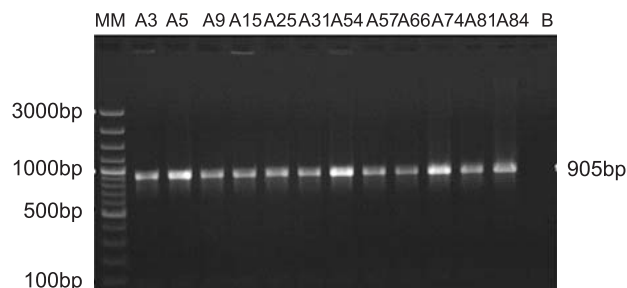


Fig. 3. Detection of *B. abortus* in samples using F4/R2 primers. Lane MM: molecular weight marker, lane A3 to A84 : samples, B: blank.

to detect as little as 0.1 pg DNA i.e. less than 100 *Brucella* cells. Due to sensitivity, specificity, rapidity of analysis and ease of automation, PCR assay could be successfully used as an important additional tool in diagnosis of brucellosis. Similar work was conducted by Gallien *et al.* (1998) who used PCR to detect *Brucella* species in the uterus, udder, spleen, lymph nodes, kidney and liver of 3 cows, which were naturally infected by brucellosis. He found 18 samples positive to PCR and 13 of them were positive in bacterial isolation. These reports confirm the sensitivity of PCR over cultural methods. Elfaki *et al.* (2005) applied PCR on serum samples for *Brucella* diagnosis and detected amplicon in 96% (24/25) samples and proposed that PCR is convenient and safe method for rapid and accurate diagnosis of brucellosis.

SUMMARY

Out of 84 samples collected from cattle and buffaloes with history of abortions and reproductive disorders 9 (10.71%) were positive by isolation and 12 (14.28%) were positive by PCR. The amplicons of 905bp and 223bp were amplified using genus specific F4/R2 and B4/B5 primers, respectively. Primers F4/R2 and B4/B5 are sensitive and specific to detect *Brucella 16S rRNA* and *bcs31* gene. Prevalence of *Brucella abortus* was 14.54% in buffaloes and 13.79% in cattle respectively by PCR. Polymerase chain reaction proved to be a better technique with regards to sensitivity, specificity, safety, rapidity of analysis and easy of automation for detection of *Brucella* species in comparison to conventional methods. *bcs31* and *16S rRNA* are conserved genes and may be good candidate for diagnosis of *Brucella* species.

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