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Molecular Epidemiology of *Brucella abortus* among Buffaloes in Western Rajasthan

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ABSTRACT

Brucellosis is a widespread reproductive disease which is clinically manifested as abortion, stillborn or weak calves, neonatal mortality, retained placenta, delayed conception, infertility, and marked reduction in milk yield of livestock. The present study was conducted to detect the presence of *Brucella abortus* in the clinical cases of reproductive disorders among the buffaloes in Western Rajasthan. A total of 33 vaginal samples including swabs (25) and discharges (8) were collected from buffaloes with a clinical history of reproductive failures, and screened using *Brucella* genus specific *bcsp31* gene based PCR followed by *Brucella abortus* species specific *IS711* based PCR. The results revealed 6.45% of the clinical samples positive for the presence of *Brucella* spp., including 8.00% of vaginal swabs and none of the vaginal discharges. All the samples detected positive in genus specific PCR were detected positive for the presence of *Brucella abortus*. The present investigation confirmed the involvement of *Brucella abortus* in the clinical cases of reproductive disorders in buffaloes in this region highlighting the need for implementation of control measures.

Keywords: Brucellosis, Buffaloes, PCR, *bcsp31*, *IS711*

One of the important diseases impacting the dairy sector in India, including Rajasthan, is brucellosis. Infection with *Brucella* in cattle and buffaloes is usually caused by biovars of *Brucella abortus*. The disease is responsible for a loss of ₹ 442.24 per cattle and ₹ 1183.65 per buffalo in India (Singh *et al*., 2015). Radostits *et al.* (2007) has attributed the economic burden posed by brucellosis to: the abortion storms in newly infected herds, a high level of retained placentae and hence endometritis or metritis resulting in reduced milk production, infertility. The infection of *Brucella* species is commonly mediated by direct contact with the placenta, fetus, fetal fluids, and vaginal discharges or byproducts (e.g., milk, meat, and cheese) from infected animals (Ferrero *et al.*, 2014).

Besides being a threat to the livestock, brucellosis is regarded as one of the most contagious bacterial zoonoses in the world which can only be managed in humans by its control and prevention in animals. The overall seroprevalence of 9.84% has been recorded among the buffaloes in Western Rajasthan by RBPT as well as i-ELISA (Priyanka *et al.*, 2018). The identification of *Brucella* at genus, species and even biovar levels has improved with the application of molecular methods, especially PCR. Molecular detection of *Brucella* spp. can be done directly on the clinical samples without the previous isolation of the organism. Thus, the speed and sensitivity of PCR assay coupled with the reduced risk to the laboratory workers, made this technique a very useful tool for the diagnosis of brucellosis. Data from India are sparse, but with the largest livestock population in the world and no brucellosis control program in place, millions of *Brucella* positive animals are likely present (Kumar, 2010). Keeping in view the importance of diagnosing the brucellosis promptly and accurately for a

better prophylaxis, prognosis, quarantine and culling of infected buffaloes to control the spread, the present study was contemplated with the objective to detect the presence of *Brucella abortus* in the clinical cases of reproductive disorders in buffaloes in Western Rajasthan by using PCR based on genus and species specific primers.

MATERIALS AND METHODS

Sample collection

In the present study, a total of 33 vaginal samples including swabs (25) and discharges (8) were collected from buffaloes with a clinical history of reproductive failures *viz*. abortion, stillborn or weak calves, neonatal mortality, retained placenta, delayed conception and/or impaired fertility. The samples were collected from the various parts of Western Rajasthan including Barmer, Bikaner, Jalore, Nagaur and Sirohi districts. All the vaginal swabs were collected in sterile vials in phosphate buffered saline (PBS) (pH 7.2) for PCR based detection. In addition to the swabs, vaginal discharges were also collected in sterile test tubes. Collected samples were transported to the laboratory in chilled conditions and stored at -20°C until further processing.

DNA extraction and PCR test

DNA was extracted from the vaginal swabs and discharges by using commercial DNA extraction kit i.e. DNeasy Blood and Tissue Kit (Qiagen) as per the manufacturer's protocol. The DNA extracted from the vaginal swabs and discharges was screened using *Brucella* genus specific PCR based on primers B4/B5 targeting the *bcsp31* gene (Baily *et al*., 1992), namely B4 (F) (TGGCTCGGTTGCCAATATCAA) and B5 (R) (CGCGCTTGCCTTTCAGGTCTG) with the approximate size of amplicon being 223 bp. This was followed by the *Brucella abortus* species specific PCR based on primers targeting the *IS711* insertion element (Bricker and Halling, 1994), namely IS711 (F) (GACGAACGGAATTTTTCCAATCCC) and IS711 (R) (TGCCGATCACTTAAGGGCCTTCAT) with the approximate size of amplicon being 498 bp.

The thermo cycling conditions for genus specific PCR were initial denaturation at 93°C for 5 min; then 35 cycles of denaturation at 90°C for 1 min, annealing at 64°C for 30 s, and extension at 72°C for 1 min; followed by final extension for 10 min at 72°C and soaking at 4°C. For species specific PCR, the conditions used were initial denaturation at 95°C for 2 min; then 35 cycles of denaturation at 95°C for 90 s, annealing at 57°C for 2 min, and extension at 72°C for 2 min; followed by final extension for 5 min at 72°C and soaking at 4°C.

For both the tests, electrophoresis was performed at 80-100 V in Tris Acetate EDTA (TAE) buffer (pH 8.0) as running buffer for 45-60 min. The gel (2 per cent agarose containing ethidium bromide) was visualized to analyze the size of bands under UV trans-illuminator using gel documentation system.

RESULTS AND DISCUSSION

For the identification of *Brucella* spp., the primers for sequences encoding *bcsp31* (B4/B5) (Al-Mariri, 2015), 16S rRNA (F4/R2) (Ciftci *et al*., 2017), 16S-23S rDNA interspace region (ITS66/ITS279) (Keid *et al*., 2007), *IS711* (IS313/IS639) (Bounaadja *et al*., 2009), *per* (bruc1/ bruc5) (Bounaadja *et al*., 2009), *omp2* (JPF/ JPR) (Kala *et al*., 2018), outer membrane proteins *omp 2b*, *omp2a* and *omp31* (Imaoka *et al*., 2007) and proteins of the *omp25/omp31* family (Martín-Martín *et al*., 2009) have been used.

The *bcsp31* gene, coding for a 31-kDa immunogenic outer membrane protein conserved among all *Brucella* spp. is the most common molecular target in clinical applications (Al Dahouk *et al*., 2014). Such a genus-specific PCR can help to avoid false-negative results in animals infected with unusual species and biovars.

In the present investigation, of the 33 clinical samples subjected to the *bcsp31* gene based genus specific PCR, total 2 samples (6.45%) were detected positive for the presence of *Brucella* spp (Fig. 1). Of these positive samples, 2 vaginal swabs out of 25 tested (8.00%) and none of the 8 samples of vaginal discharges tested were positive for *Brucella* spp. The same *bcsp31* gene based PCR was utilized by Patel *et al*. (2017) to screen samples in Gujarat and they found 1 out of 45 vaginal samples and 1 out of 5 vaginal discharges from buffaloes positive for *Brucella* spp.

Several studies have described PCR assays that make use

of the specific occurrence of the multiple insertion element IS*711* which is stable in both number and position in the *Brucella* chromosomes as a target (Santis *et al*., 2011). Hence, in the present study, primers targeting *IS711* gene were employed for *Brucella abortus* species specific PCR.

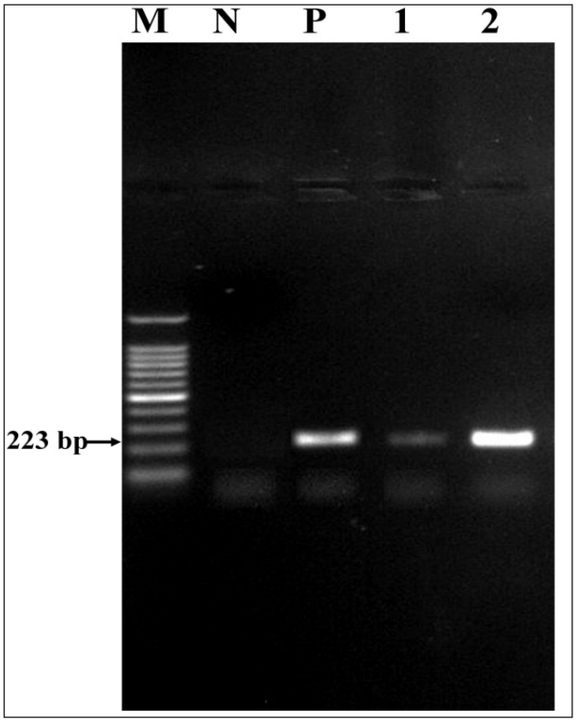

Fig. 1: PCR based on genus specific primers, targeting the *bcsp31* gene. PCR positive samples (lane 1, 2) show amplicon of approximately 223 bp. N is negative control. P is positive control. M is 100 bp marker

The 2 clinical samples i.e. vaginal swabs which were found to be positive in genus specific PCR, were also subjected to the *IS711* based species specific PCR. Both the samples detected positive in genus specific PCR i.e. 6.45% (2 out of 33) of the vaginal samples including 8.00% (2 out of 25) of the swabs and none of the discharges, were detected positive for the presence of *Brucella abortus* (Fig. 2).

The PCR assay for detection of *Brucella* DNA using *bcsp31* target gene and *IS711* locus was conducted by Garshasbi *et al*. (2014) which showed that an amplicon of 223 bp was obtained in 73.8 % (133/180) of the tested sera using primers (B4/B5) and an amplicon of 498 bp was obtained in 63.8% (115/180) of the samples using *Brucella abortus*-specific primers derived from a locus adjacent to the 3'-end of *IS711*. But, in another study conducted by Patel *et al*. (2015), all the samples (7 out of 33) from aborted buffaloes which yielded *Brucella* in genus specific PCR were confirmed as *Brucella abortus* in species specific PCR based on *IS711* as well. Similarly, when Karthik *et al*. (2014) used the same primers i.e. *bcsp31* and *IS711* for the detection and identification of *B. abortus* in blood samples (n=370) of cattle from three states *viz*. Uttar Pradesh, Uttarakhand and Tamil Nadu, a total of 56 samples (15.03 %) were detected as positive by both the PCRs.

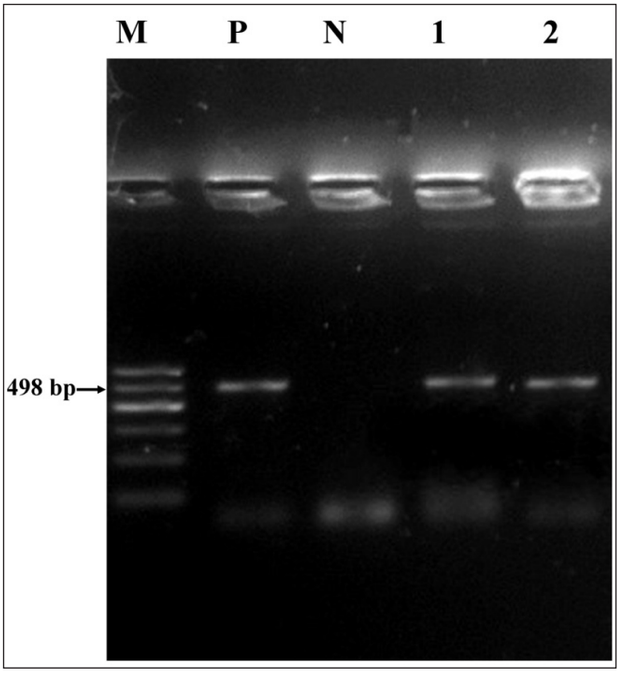

Fig. 2: PCR based on species specific primers, targeting the *IS711* insertion element. PCR positive samples (lane 1,2) show amplicon of approximately 498 bp. N is negative control. P is positive control. M is 100 bp marker

In the present study, we were able to detect *Brucella abortus* in the vaginal swabs and vaginal discharges from buffaloes. However, there may be a number of reasons why the PCR assay for *B. abortus* has not yet surpassed the serological and bacteriological methods. The stage of infection may influence the number and location of bacteria. Secondly, the presence of large amounts of bovine genomic DNA may have inhibitory effects on

the PCR assay. As narrated and concluded by previous workers, each type of clinical sample has inherent and unique difficulties for adequate sample preparation besides the difference in detection limits as per the DNA extraction protocol. Hence, these might have attributed in limited number of samples yielding positive results during the present work on the PCR based detection of *Brucella abortus* from the clinical samples of buffaloes. Moreover, isolates of *Brucella* from water buffalo are less virulent compared to those from cattle (Adesiyun *et al*., 2011) suggesting some degree of resistance in buffalo towards *Brucella abortus*. Even in buffalo herds heavily infected with *Brucella abortus*, 20% of the animals remain negative by serologic tests and presumably uninfected at all times (Zimmer, 2014).

CONCLUSION

As per the findings of the present study, it can be concluded that there is a substantial involvement of *Brucella abortus* in the clinical cases of reproductive disorders among the buffaloes in Western Rajasthan. The detection of *Brucella abortus* in the vaginal swabs and discharges is alarming because in endemic herds, the birth of infected but apparently healthy calves may lead to uncontrolled spread of disease. Thus, there is an urgent need to make the farmers and veterinary professionals aware and to raise prevention and eradication campaigns to control the disease in Western Rajasthan and entire India as a whole.

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