



## A Study on the Molecular Epidemiology of *Brucella abortus* among Cattle in Western Rajasthan

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### ABSTRACT

Bovine brucellosis is usually caused by biovars of *Brucella abortus*. It is one of the important diseases impacting the dairy sector in India, including Rajasthan, which is often neglected because of its endemicity and often persists in the poorest and most vulnerable populations. The present study was conducted to assess the prevalence of *Brucella abortus* in the cases of reproductive disorders such as abortion, stillborn or weak calves, neonatal mortality, retained placenta, delayed conception and infertility among the cattle in Western Rajasthan. A total of 87 vaginal samples including swabs (64) and discharges (23) were collected from cattle with a clinical history of reproductive failures, and screened using *Brucella* genus specific *bcs31* gene based PCR followed by *Brucella abortus* species specific *IS711* based PCR. The results revealed 12.64% of the clinical samples positive for the presence of *Brucella* spp., including 12.50% of vaginal swabs and 13.04% of the vaginal discharges. All the samples detected positive in genus specific PCR were detected positive for the presence of *Brucella abortus*. The present study confirmed the considerable involvement of *Brucella abortus* as a major etiological agent in the clinical cases of reproductive disorders in cattle highlighting the urgent need for implementation of strict measures to control the disease in Western Rajasthan.

**Keywords:** Brucellosis, Cattle, PCR, *bcs31*, *IS711*

Bovine brucellosis is usually caused by biovars of *Brucella abortus*. It is one of the important diseases impacting the dairy sector in India, including Rajasthan, which is often neglected because of its endemicity. The state of Rajasthan has over 11 million heads of cattle which account for around 10% of the cattle population of the country (Sharma *et al.*, 2017). For Western Rajasthan, livestock is the major source of livelihood for the poor but the cattle breeding is losing ground due to lack of economic viability. The disease is essentially an economic threat and is responsible for a loss of ₹ 442.24 per cattle in India (Singh *et al.*, 2015), The overall seroprevalence of 13.89% and 15.28% by RBPT and i-ELISA, respectively has been recorded in Western Rajasthan (Priyanka *et al.*, 2017).

This widespread reproductive disease commonly causes

abortion, death of young ones, stillbirth, retained placenta or birth of weak calves, delayed calving, male infertility, and marked reduction in milk yield (Garofolo *et al.*, 2016; Arif *et al.*, 2017; Curro *et al.*, 2012; Priyanka *et al.*, 2019a & b). 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 (Godfroid *et al.*, 2005; Ferrero *et al.*, 2014; Priyanka *et al.*, 2018). Besides, being one of the most contagious bacterial zoonoses in the world, brucellosis in humans can only be managed by its control and prevention in animals, thus entailing the need for its urgent and accurate diagnosis and implementation of control measures in livestock as well. A number of PCR methods developed for the detection of *Brucella* are more and more used in the diagnosis of brucellosis owing to



their more sensitivity than conventional culture methods and more specificity than serological methods (Al Dahouk *et al.*, 2013).

Keeping in view the importance of diagnosing the brucellosis promptly and accurately for a better prophylaxis, prognosis, quarantine and culling of infected cattle to control the spread, the present study was contemplated with the objective to assess the prevalence of *Brucella abortus* in cattle 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 87 vaginal samples including swabs (64) and discharges (23) were collected from cattle 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, Jaisalmer, Jalore, Nagaur and Sirohi districts. All the vaginal swabs were collected in sterile vials in phosphate buffer 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 *bcs31* 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) (GACGAACGGAATTTTCCAATCCC) 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.

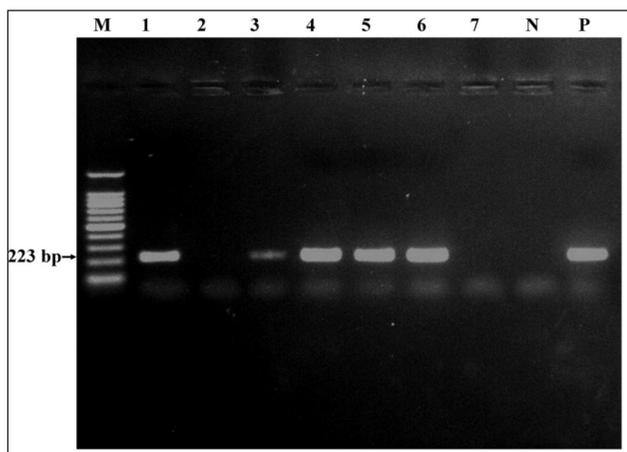
## RESULTS AND DISCUSSION

For the identification of *Brucella* spp., the primers for sequences encoding *bcs31* (B4/B5) (Baily *et al.*, 1992), 16S rRNA (F4/R2) (Romero *et al.*, 1995), 16S-23S intergenic transcribed spacers (ITS) (Rijpens *et al.*, 1996; Bricker, 2000), 16S-23S rDNA interspace region (ITS66/ITS279) (Keid *et al.*, 2007), *IS711* (IS313/IS639) (Hénault *et al.*, 2000), *per* (*bruc1/bruc5*) (Bogdanovich *et al.*, 2004), *omp2* (JPF/JPR) (Leal-Klevezas *et al.*, 1995), outer membrane proteins (Imaoka *et al.*, 2007) and proteins of the *omp25/omp31* family (Vizcaino *et al.*, 2004) have been used.

The *bcs31* gene, coding for a 31-kDa immunogenic outer membrane protein conserved among all *Brucella* spp. is the most common molecular target in clinical applications (Baily *et al.*, 1992). Such a genus-specific PCR can help to avoid false-negative results in individuals infected with unusual species and biovars.

In the present investigation, of the 87 clinical samples subjected to the *bcs31* gene based genus specific PCR, total 11 samples (12.64%) were detected positive for the presence of *Brucella* spp (Fig 1). Of these 11 positive samples, 8 vaginal swabs out of 64 tested (12.50%) and 3 vaginal discharges out of 23 tested (13.04%) were positive for *Brucella* spp. The same *bcs31* gene based PCR was

utilized by Patel *et al.* (2017) to screen samples in Gujarat and they found 1 out of 73 vaginal samples and 2 out of 8 vaginal discharges from cattle positive for *Brucella* spp.



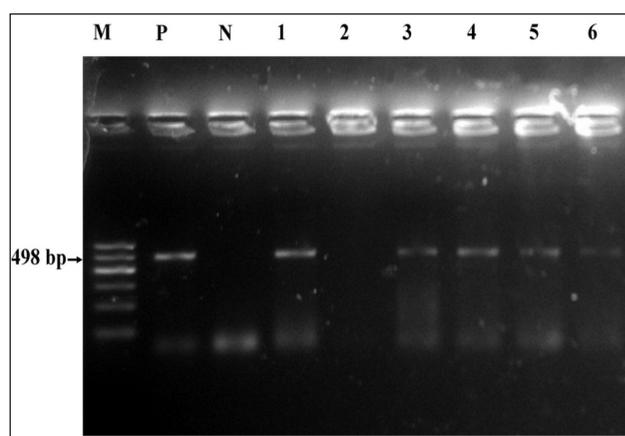
**Fig. 1:** PCR based on genus specific primers, targeting the *bcs31* gene. Lane 1 to 7 are test samples. PCR positive samples (lane 1,3,4,5,6) show amplicon of approximately 223 bp. N is negative control. P is positive control. M is 100 bp marker

Several studies have described PCR assays that make use of the specific occurrence of the multiple insertion element *IS711* which was described by Halling *et al.* (1993) and is stable in number and position in the *Brucella* chromosomes (Bricker and Halling 1994; Ohishi *et al.*, 2004; Ocampo-Sosa *et al.*, 2005). Hence, in the present study, primers targeting *IS711* gene were employed for *Brucella abortus* species specific PCR.

The 11 clinical samples including 8 vaginal swabs and 3 vaginal discharges which were found to be positive in genus specific PCR, were also subjected to the *IS711* based species specific PCR. All the samples detected positive in genus specific PCR i.e. 12.64% (11 out of 87) of the vaginal samples including 12.50% (8 out of 64) of the swabs and 13.04% (3 out of 23) of the discharges, were detected positive for the presence of *Brucella abortus* (Fig 2).

The PCR assay for detection of *Brucella* DNA using *bcs31* 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. *bcs31* 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. Lane 1 to 6 are test samples. PCR positive samples (lane 1,3,4,5,6) show amplicon of approximately 498 bp. N is negative control. P is positive control. M is 100 bp marker

## CONCLUSION

Thus, as per the findings of the present study, it can be concluded that there is a substantial involvement of *Brucella abortus* as a major etiological agent in the clinical cases of reproductive disorders among the cattle in Western Rajasthan. Such a high prevalence in the vaginal swabs and discharges is alarming because in herds where brucellosis is already endemic, the birth of weak and also healthy, but infected calves may occur and there is uncontrolled spread of disease. Thus, there is an urgent need to make the farmers and veterinary professionals aware and to implement strict control measures to prevail over the dispersal of *Brucella* infection and control the disease in Western Rajasthan and entire India as a whole.



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