RESEARCH ARTICLE

Detection of *Brucella* Associated with Bovine Abortions in an Organized Dairy Farm

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Abstract

The *Brucella abortus* is an important bacterial pathogen responsible for reproductive losses in cattle and buffaloes. In females, the disease is manifested as late-term abortion (commonly during the last trimester), retained placenta, reduced fertility and repeat breeding or even stillbirths or birth of weak calves. The present study screened a total of 12 abortion-associated cases samples, including blood, placental tissues, and foetal stomach contents, from both cattle and buffaloes from an organized dairy farm in Punjab. Serum samples were screened for Brucella antibodies using the Rose Bengal Plate Test (RBPT) and the placental and foetal samples were subjected to bacterial isolation. Genomic DNA extracted directly from clinical samples and from culture isolates was subjected to genus-specific PCR targeting the *bcsp31* gene. Samples positive by genus-specific PCR were further characterized using Bruce-ladder multiplex PCR. Of the 12 serum samples tested, 7 were positive by RBPT. Direct genus-specific PCR detected Brucella DNA in 11 samples, yielding the expected 223-bp amplicon. Four Brucella isolates were successfully recovered through culture and subsequently confirmed by genus-specific PCR. Bruce-ladder multiplex PCR confirmed all genus-specific PCR-positive samples as *Brucella abortus*, producing characteristic amplicons of 152, 450, 587, 794, and 1682 bp. The detection of *Brucella abortus* in aborted samples from cattle and buffaloes through serological, molecular, and bacteriological methods underscores the importance of an integrated diagnostic approach for accurate diagnosis and effective control of brucellosis.

Key words: Abortions, *Brucella abortus,* Diagnosis, Isolation, PCR

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Introduction

Brucella abortus is a facultative intracellular, Gram-negative bacterium which causes bovine brucellosis. The disease is a globally important zoonotic disease that causes significant reproductive losses in cattle and buffaloes (Corbel, 2006). The disease is endemic in many developing countries, including India, imposing serious economic and public health burdens due to abortion, stillbirths, infertility, reduced milk production, and trade restrictions (Godfroid *et al.,* 2011; Upadhyay *et al.,* 2019). In infected female animals, *Brucella abortus* shows a marked tropism for the gravid uterus, particularly during late gestation, owing to the presence of sugar erythritol and other growth-promoting factors in placental tissues (Carvalho *et al.,* 2023). Consequently, infection is most commonly manifested as abortion during the last trimester, retained placenta, metritis, birth of weak calves, or stillbirths (Poester *et al.,* 2013). Aborted foetuses, placental membranes, uterine discharges, and milk contain high concentrations of the organism, facilitating rapid spread within herds and posing a significant zoonotic risk to farm workers and veterinarians (Seleem *et al.,* 2010).

Diagnosis of brucellosis remains challenging due to the variable clinical presentation and the occurrence of subclinical infections. Bacterial culture is considered the gold standard for confirmation; however, it is time-consuming, risk group 3 pathogen requires biosafety level-3 facilities, and poses a substantial risk of laboratory-acquired infection (Ciftci *et al.,* 2017). Serological tests such as the Rose Bengal Plate Test (RBPT) are widely used for herd screening due to their simplicity and low cost, but they may yield false-positive or false-negative results owing to cross-reactivity or low

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antibody titres, particularly in early or chronic infections (Nielsen and Yu, 2010). Molecular diagnostic techniques, especially polymerase chain reaction (PCR)-based assays, are rapid and show more sensitive, and specific detection of Brucella DNA directly from clinical samples (Liu and Jiang, 2024). Genus-specific PCR targeting conserved genes such as *bcsp31* enables reliable detection of *Brucella* spp., while multiplex assays such as Bruce-ladder PCR facilitate species-level differentiation, including identification of *B. abortus* (Lopez-Goni *et al.,* 2008). These methods are particularly valuable in abortion outbreaks, where prompt and accurate diagnosis is critical for implementing effective control measures.

Given the occurrence of abortion and stillbirths in organized dairy farms and the endemic nature of brucellosis in the region, the present study was undertaken to screen abortion-associated cases in cattle and buffaloes for *Brucella abortus* using an integrated diagnostic approach involving serology, bacteriological isolation, and molecular techniques. The study aims to generate confirmatory evidence of infection at the farm level and to highlight the importance of combining conventional and molecular diagnostics for effective surveillance and control of bovine brucellosis.

Materials and Methods

Study Design and Sample Collection

The present study was conducted to detect *Brucella abortus* in abortion-associated cases of cattle and buffaloes maintained at an organized dairy farm in Punjab, India. A total of 12 clinical cases involving abortion or stillbirth were included in the study period from May 2025 to October 2025. The animals comprised both cattle ((Jersey and Friesian crossbreds) and buffaloes (Murrah and Nili-Ravi) aged between 1.6 and 7.8 years. Detailed reproductive history, including stage of gestation at abortion, were also recorded. Among the 12 cases, 10 abortions occurred during the last trimester, one during the second trimester, and one case resulted in stillbirth.

From each case, multiple clinical samples were collected aseptically, including whole blood for serum separation, placental tissues, and foetal stomach contents. Blood samples were collected via jugular vein puncture and allowed to clot at room temperature, followed by serum separation through centrifugation. Placental tissues and foetal stomach contents were collected in sterile containers, transported to the laboratory under cold chain conditions, and processed immediately or stored at –20 °C until further analysis.

Serological Screening

Serum samples were screened for the presence of anti-Brucella antibodies using the Rose Bengal Plate Test (RBPT) following standard protocols. Briefly, equal volumes of test serum and Rose Bengal antigen (30 µL) were mixed on a clean glass plate and gently mixed for few minutes. Agglutination was observed visually, and samples showing definite clumping were considered positive. Positive and negative controls were run along with the test samples.

Bacteriological Isolation

Placental tissues and foetal stomach contents were processed for bacteriological culture. Samples were homogenized in sterile phosphate-buffered saline under biosafety precautions. The homogenates were inoculated onto Brucella agar plates both with and without selective supplements. The inoculated plates were incubated at 37 °C under aerobic conditions, with 5-10% CO_2, and observed for up to 7-10 days. Suspected colonies showing typical Brucella morphology were subcultured to obtain pure isolates and preserved for further molecular confirmation.

DNA Extraction and Genus-Specific PCR

Genomic DNA was extracted directly from clinical samples (placental tissues and foetal stomach contents) as well as from culture-confirmed isolates using a Qiagen Kit according to the manufacturer's instructions (Catalog no.69504). The quality and concentration of extracted DNA were assessed using spectrophotometric methods and eluted DNA (50 µL) was stored at –20° C until PCR analysis. Bruce-ladder multiplex PCR was performed to detect *Brucella* species, as described by Bertu *et al.* (2022) with some modifications. All the extracted DNA samples were subjected to genus-specific PCR targeting the *bcsp31* gene, a conserved gene among *Brucella* species using specific primers B4(F) 5' TGG CTC GGT TGC CAA TAT CAA 3' and B5(R) 5' CGC GCT TGC CTT TCA GGT CTG 3' (Bricker and Halling, 1994). PCR amplification was carried out in a total of 25 µL PCR reaction mixture consisted of 2X PCR master mix (Qiagen) 12.5 µL, primers sets (10 pmol) 1.0 µL each, 4 µL DNA template, and 6.5 µL NFW. The cycling conditions included initial denaturation 95 °C for 5 min, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (60 °C for 45 s), and extension (72 °C for 45 s), with a final extension at 72 °C for 10 min. PCR products were resolved by agarose gel electrophoresis and visualized under UV illumination after ethidium bromide. Samples yielding the expected 223-bp amplicon were considered positive for *Brucella* genus.

Bruce-Ladder Multiplex PCR

Samples positive by genus-specific PCR were further subjected to Bruce-ladder multiplex PCR for species-level identification. This multiplex assay was performed using a set of primers targeting multiple loci specific to different *Brucella* species (Lopez-Goni *et al*., 2008). The PCR reaction conditions were optimized according to established protocols. Briefly, the 25 µL PCR reaction consisted of 12.5 µL of 2x Master Mix (Qiagen), 7.5 µL cocktail primer (10 pmol each), 3 µL DNA template, and 2 µL nuclease-free water. PCR was performed in a Vertis thermal cycler under the following conditions: 95 °C for 15 min, followed by 30 cycles of denaturation (94 °C for

30 s), annealing (64 °C for 45 s), and extension (72 °C for 1 min), with a final extension at 72 °C for 10 min. Gel electrophoresis was performed using 1.5% agarose (Himedia) stained with ethidium bromide (Himedia MB074-10ML) in TBE buffer for PCR product analysis. Amplified products were separated on agarose gel electrophoresis, and banding patterns were analyzed to identify species-specific amplicons. Characteristic band profiles were used to confirm *Brucella abortus* in both clinical samples and culture isolates.

Data Interpretation

Results obtained from serological testing, bacteriological culture, and molecular assays were compiled and compared. The concordance between RBPT, PCR-based detection, and culture isolation was assessed descriptively. Detection of *Brucella abortus* by one or more diagnostic methods was used to confirm infection.

Results and Discussion

Serological and Bacteriological Findings

Serum samples from all 12 animals were screened for anti-Brucella antibodies using the Rose Bengal plate test (RBPT). Out of the 12 serum samples tested, 7 (58.3%) showed visible agglutination and were recorded as positive, while 5 (41.7%) samples were negative. Although RBPT is widely employed for rapid herd-level screening, the lower detection rate (58.3%) compared to PCR observed in this study highlights its limitations. False-negative results in RBPT may occur in early stages of infection, chronic cases, or due to immunological tolerance, while false positives can arise from cross-reacting organisms (Nielsen and Yu, 2010).

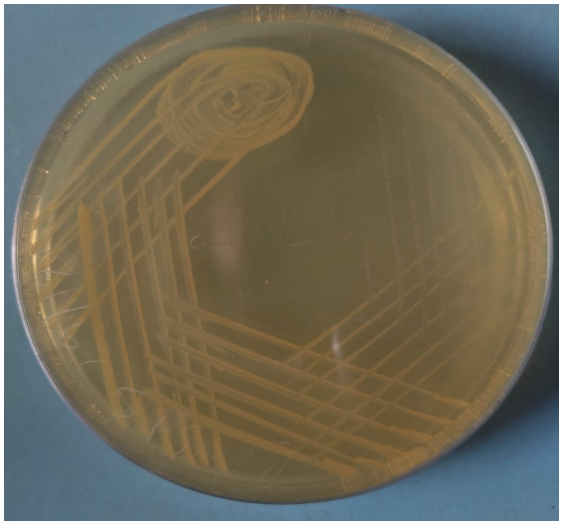

Fig. 1: Isolates of Brucella typical morphological characteristics successfully subculture to obtain pure isolates of *B. abortus* on Brucella agar.

Placental tissues and foetal stomach contents were cultured on Brucella agar with and without selective supplements. Four samples (33.3%) yielded bacterial growth consistent with *Brucella* spp. colonies exhibiting typical morphological characteristics and were successfully subcultured to obtain pure isolates (Islam *et al.,* 2019) (Fig. 1). No growth was observed in the remaining samples during the incubation period. Bacteriological isolation yielded viable *Brucella* organisms from only four cases, reflecting the inherent difficulties associated with culturing *Brucella* spp. The low isolation rate may be attributed to intermittent shedding, low bacterial load, sample quality, or prior antibiotic exposure (Dawood *et al.,* 2023). Nonetheless, isolation remains critical for definitive diagnosis, epidemiological studies, and strain characterization.

Genus-Specific PCR Detection

Direct PCR analysis detected Brucella DNA in 11 out of 12 cases (91.7%), producing the expected 223 bp amplicon (Fig. 2). All four culture-positive isolates were also confirmed as *Brucella* spp. by genus-specific PCR. Genus-specific PCR targeting the *bcsp31* gene demonstrated the highest detection rate (91.7%), detecting *Brucella* DNA directly from clinical samples, including those that were negative by serology and culture. This finding supports earlier reports emphasizing the superior sensitivity of PCR in detecting *Brucella* in abortion materials, particularly when bacterial viability is compromised or when antimicrobial treatment has been administered prior to sampling (Lita *et al.,* 2025). The ability of PCR to rapidly identify infected cases without the need for prolonged incubation or high-level biosafety facilities makes it particularly valuable in outbreak investigations. The discrepancy between serological and molecular findings in the present investigation reinforces the need for confirmatory tests when RBPT is used as a primary screening tool. However, recently isothermal assays targeting *bcsp31* gene have been developed which might be helpful in detection of *Brucella* infection in low cost setting with comparable sensitivity and specificity (Shukla *et al .,* 2023).

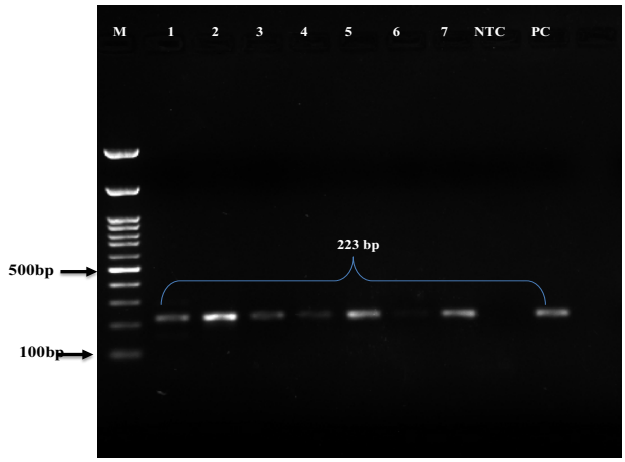

Fig. 2: Fig.2 Screening of isolate sample for brucella genus using Conventional PCR *bcsp31* gene in house developed primer 223-bp amplicon: Lane M: 100 bp plus ladder Marker, Lane 1-7 sample, lane 8: Negative template control (NTC) , Lane 9 : Positive control of *B. abortus* (PC)

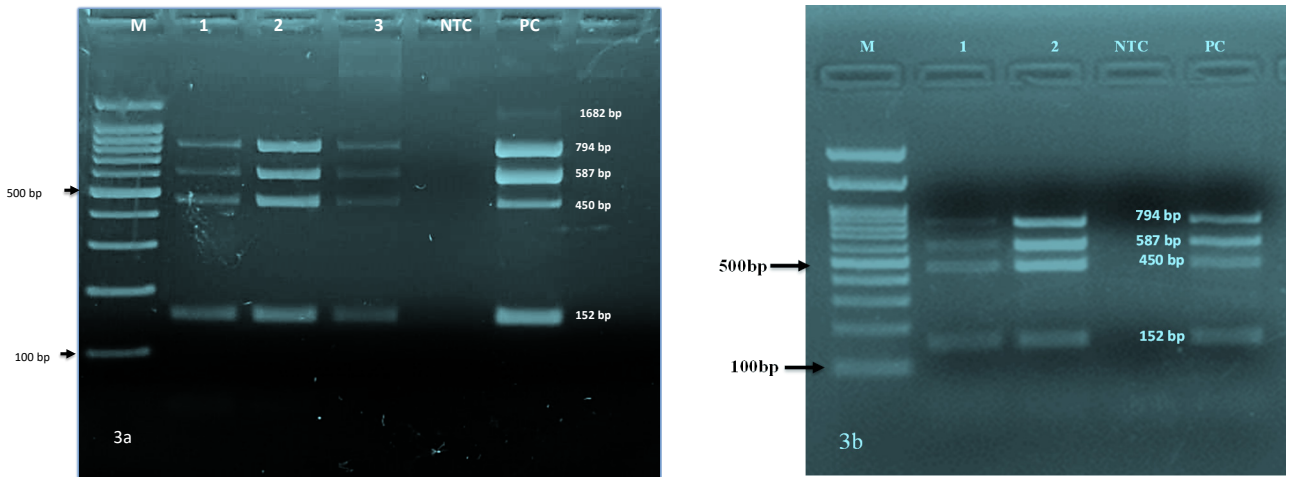

Fig. 3: Molecular detection of *Brucella* species by Bruce-ladder multiplex PCR assay: **3a.** Abortion screening: Lane M: 100 bp plus ladder Marker, Lane 1-3 sample, lane 4: Negative template control (NTC), Lane 5: Positive control of *B. abortus* (PC). **3b.** culture Isolate: Lane M: 100 bp plus ladder Marker, Lane 1-2 Isolate, Lane 3: Negative template control (NTC), Lane 4: Positive control of *B. abortus* (PC).

Species Identification by Bruce-Ladder Multiplex PCR

All samples positive by genus-specific PCR were further analyzed using Bruce-ladder multiplex PCR. The multiplex PCR assay confirmed all 11 genus-positive samples as *Brucella abortus*, based on the presence of characteristic amplicons of 152, 450, 587, 794, and 1682 bp (Fig. 3 a, b). The four culture-confirmed isolates were also subjected to Bruce-ladder multiplex PCR for species-level identification. All isolates produced the *Brucella abortus*-specific banding pattern, yielding amplicons of 152, 450, 587, 794, and 1682 bp, thereby conclusively identifying them as *Brucella abortus*.

Brucella abortus remains a major cause of reproductive failure in cattle and buffaloes, particularly in endemic regions such as India. The present study investigated abortion- and stillbirth-associated cases in an organized dairy farm and confirmed the involvement of *B. abortus*. The Bruce-ladder multiplex PCR enabled clear differentiation of *B. abortus* from other *Brucella* species, supporting previous studies that advocate its use as a robust and reliable tool for species-level identification (Lopez-Goni *et al.,* 2008). Accurate species identification is essential for understanding transmission dynamics, implementing appropriate control strategies, and assessing zoonotic risk at the farm and regional levels.

Conclusion

The present study reports the carriage of *B. abortus* in large ruminants on a dairy farm with episodes of abortion associated stillbirth in dairy sector. The findings underscore the need for comprehensive surveillance studies on brucellosis in farm. Additionally, public health initiatives should emphasize educating animal rearers about potential zoonotic risks and the necessary precautions to minimize zoonotic transmission.

Acknowledhement

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