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Serological, Isolation and Molecular Studies on Brucellosis in an Organized Farm, Jammu and Kashmir, India

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ABSTRACT

The present study was conducted in an organized farm of Jammu region, Jammu and Kashmir state having history of late-term abortions in cattle and buffaloes to elucidate the role of brucellosis in causing abortions on the farm. The farm had 46 animals (23 adults, 15 heifers and 9 calves). The farm was positive in herd test (milk ring test). Serological tests viz., Rose Bengal plate test (RBPT) and serum agglutination test (SAT) were conducted on 30 serum samples and 11 samples were found positive in both tests. Five vaginal swabs from abortion cases were processed for isolation and 3 isolates of *Brucella* were obtained which on further characterization by biochemical tests, genus-specific PCR and species-specific PCR were identified as *B. abortus* biovar 1. As brucellosis is an anthropozoonoses, the high prevalence of brucellosis in livestock of farm demands the surveillance of disease in humans working on the farm and necessitates the control of disease on the farm.

Keywords: Brucellosis, isolation, Jammu and Kashmir

Brucellosis is an infectious disease responsible for huge economic losses to livestock industry. The infection leads to late-term abortions in animals along with retention of placenta and reduced milk yield inflicting financial losses. Brucellosis is caused by bacterium of genus *Brucella* having six classical species viz., *Brucella abortus* (cattle, buffaloes), *B. melitensis* (sheep, goats), *B. suis* (pigs), *B. ovis* (sheep, goats), *B. canis* (dogs) and *B. neotomae* (desert wood rat) depending upon host specificity, pathogenicity and phenotypic differences. The infection from animals to humans may get transmitted and infection in humans is manifested in the form of biphasic fever and involvement of bones and joints (Seleem *et al.*, 2010). Every year around 500000 human brucellosis cases are reported worldwide (Cutler *et al.*, 2005).

The routine diagnosis of brucellosis in cattle and buffaloes is done by serological tests namely Rose Bengal plate Test (RBPT) and serum agglutination test (SAT) whereas isolation of bacterium is considered as gold standard test (Boral *et al.*, 2009; Pathak *et al.*, 2016). Polymerase chain reaction (PCR) targeting genus and species have also been employed in diagnostic strategies (Singh *et al.*, 2010; Yu and Nielsen, 2010).

The present study was conducted in an organized farm of Jammu region, Jammu and Kashmir state having history of late-term abortions in cattle and buffaloes to elucidate the role of brucellosis in causing abortions on the farm.

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MATERIALS AND METHODS

An organized bovine (cattle and buffaloes) farm of Jammu region of Jammu and Kashmir state, India, having history of third-trimester abortions was studied to investigate the role of brucellosis in causing abortions. The farm had 46 bovines (23 adults, 15 heifers and 9 calves). To identify the status of herd to be positive for brucellosis, pooled milk samples of farm were tested periodically by milk ring test (MRT) (OIE, 2018). As MRT was positive for each tested sample, the individual animal serum samples from animals were collected. Serum samples (n=30) from 21 adults, 8 heifers and 1 calf were collected and tested by RBPT. RBPT was done as per the protocol of OIE (OIE, 2018). RBPT positive samples were tested by SAT to confirm positive animals. The antigens for RBPT and SAT were procured from Division of Biological Products, Indian Veterinary Research Institute, Bareilly.

For isolation of *Brucella*, post-partum vaginal swabs from 5 abortion cases were collected and processed for isolation of *Brucella* as per the protocol of Alton *et al.* (1975) using tryptic soy agar supplemented with 5% horse serum and Farrell's supplement (HiMedia, India). The vaginal swabs were streaked on preformed tryptic soy agar plates and incubated at 37°C at 5% CO_2. Plates were checked for *Brucella* growth from 4th day onwards to 14 days. The shiny, glistening, smooth colonies of 2-3 mm size which were translucent and pale honey in indirect sunlight were presumed to be *Brucella* colonies. The suspected *Brucella* colonies were further purified and subjected to biochemical tests viz., Gram staining, catalase, oxidase, urease, nitrate reductase and agglutination with polyclonal sera for confirmation.

Phenotypically identified *Brucella* isolates were subjected to genus-specific PCR (BCSP 31 gene) to confirm *Brucella* (B4 primer: 5'–TGGCTCGGTTGCCAATATCAA; B5 primer: 5'-CGCGCTTGCCTTTCAGGTCTG) (Baily *et al.*, 1992). DNA of isolates was extracted by QIAamp DNA mini kit (Qiagen, Germany) as directed in the manufacturer guidelines. The 25 µl PCR reaction mixture was comprised of 1x DreamTaq Green PCR master mix (Thermoscientific, USA), primer 10 pmol each and template 2 µl; PCR conditions were 94°C for 5 min, 25 cycles of 94°C for 45 s, 53.5°C for 45 s and 72°C for 1 min followed by 72°C for 5 min. The positive isolates yielded 223 bp PCR product in 1.5% agarose gel electrophoresis.

The confirmed *Brucella* isolates were further subjected to species-specific PCR to identify the species to be *B. abortus* (Bricker and Halling, 1994). Twenty-five microliter PCR reaction mixture was comprised of 1x PCR Buffer (Promega, USA), $MgCl_2$ – 1.5mM, dNTP each – 200 µM, Primer each – 0.4 µM (*B. abortus* primer: 5'– GACGAACGGAATTTTTCCAATCCC–3'; IS711 primer: 5'–TGCCGATCACTTAAGGGCCTTCAT – 3'), Taq polymerase – 1 unit and Template 2 µl. PCR conditions were 95°C for 2 min, 30 cycles of 95°C for 30 sec, 52°C for 30 sec and 72°C for 45 sec, and 72°C for 5 min. The isolates producing 498 bp PCR product were conformed to be *B. abortus*.

B. abortus isolates were subjected to biovar identification tests viz., growth in presence of CO_2, H_2S production, urease test, growth in presence of dyes (thionin and basic fuchsin) and agglutination with A or M – monospecific sera (Alton *et al.*, 1975).

RESULTS AND DISCUSSION

Serological study: On analysis of 30 serum samples by RBPT, 11 samples were found positive. RBPT positive samples were also positive in SAT (≥80 IU). Among 11 positive samples, 8 were of adults (8/21; 38.09%) and 3 (3/8; 37.5%) were of heifers. Thus, the seroprevalence of brucellosis in the farm was 11/30 (36.67 %) indicating high prevalence of infection in the herd.

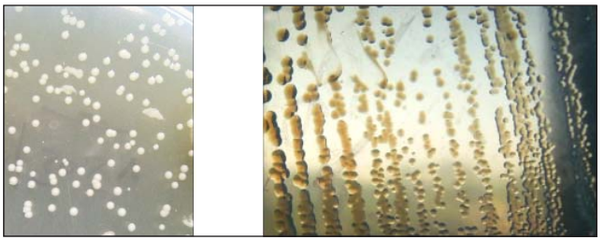
Brucellosis is an endemic disease in India with its prevalence varying from region to region. Numerous studies have been conducted in animals and humans to determine its prevalence. In a nationwide survey, Isloor *et al.* reported the seroprevalence of brucellosis in cattle to be 1.9 % and in buffalo to be 1.8 % (Isloor *et al.*, 1998). In another nationwide survey, Renukaradhya *et al.* (2002) reported the prevalence to be 5 % in cattle and 3% in buffaloes. However, the high prevalence rates were obtained by Chand and Chhabra in bovines of Haryana (22.34 %) and Punjab (34.15 %) (Chand and Chhabra, 2013). In Goa, the prevalence in cattle is reported to be 27.02 % (Pathak *et al.*, 2016). The high prevalence in these studies could be due to sampling from animals having history of abortion, retention of placenta or other reproductive problems.

There have been limited studies on bovine brucellosis in Jammu region of Jammu and Kashmir state. The

nationwide conducted survey reported the prevalence of brucellosis in cattle to be 1.2 % (Renukaradhya *et al.*, 2002). The other studies have reported the prevalence of brucellosis in cattle to be 1.88% while in buffaloes to be 4.38% (Khajuria *et al.*, 2014a; 2014b). In present study, the prevalence of brucellosis in herd was 36.67%, much higher than the reported prevalence rates, as the samples were from a single herd positive for brucellosis and it is well reported that brucellosis is a herd disease and disease once enters into the herd progresses in other animals of the herd.

Isolation and molecular study

On analysis of 5 vaginal swabs collected from abortion cases, 3 *Brucella* isolates were obtained (Fig. 1a, 1b). In biochemical testing, the isolates were Gram negative short coccobacilli, catalase positive, oxidase positive, urease positive and nitrate reductase positive. In genus-specific PCR, the isolates produced 223 bp size confirming *Brucella* genus (Fig. 2).



(a) **(b)**

Fig. 1(a): Shiny, glistening, smooth, convex colony of *Brucella*; **(1b)** Pale honey and translucent *Brucella* colony in indirect sun light

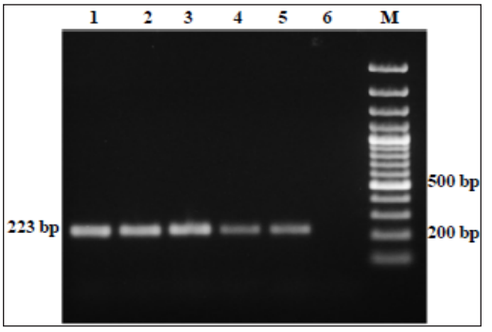


Fig. 2: PCR amplification to identify *Brucella genus*

M: Marker, 1: *B. melitensis,* 2: *B. abortus,* 3-5: Field isolates, 6: Negative control.

In species-specific PCR, the isolates produced 498 bp product size indicating the isolates to be *B. abortus* (Fig. 3).

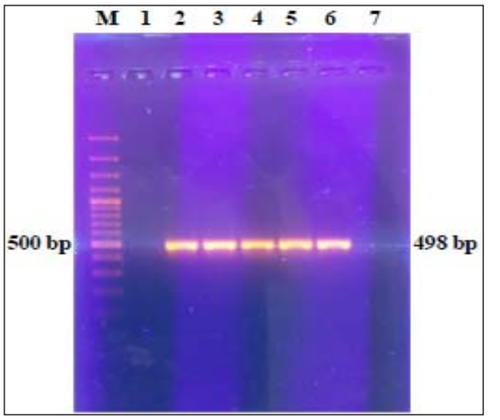


Fig. 3: PCR amplification to identify *B. abortus* species

M: Marker, 1: B. *melitensis* positive control, 2: B. *abortus* positive control, 3-6: Field isolates, 7: Negative control.

In biovar analysis, the isolates were CO_2 dependent, H_2S producers, grew at 1:25000, 1:50000 and 1:100000 dilutions of basic fuchsin but not of thionin, and agglutinated with A-monospecific serum but not with that of M-monospecific, indicating the isolates to be *B. abortus* biovar 1. *B. abortus* biovar 1 is the most widely spread biovar of *B. abortus* and is isolated from Punjab and Himachal Pradesh states of country (Chahota *et al.*, 2003; Jain *et al.*, 2013; Kaur *et al.*, 2006; Verma *et al.*, 2000). Isolation is the confirmatory test used in diagnosis of brucellosis and isolation from abortion cases confirms the active infection of *Brucella* in the herd. Further, biovar identification will help in understanding of epidemiology of disease in the region. High seroprevalence of brucellosis and isolation of *Brucella* from abortion cases in herd clearly indicated the role of brucellosis in causing abortions in the farm.

CONCLUSION

Brucellosis was prevalent in high proportion in the studied farm. It necessitates the detection of brucellosis in the working staff on the farm and demands interventions to prevent the transmission of disease in animals and humans on the farm.

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