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Seroprevalence and Molecular Detection of Bovine Brucellosis

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ABSTRACT

Background: Brucellosis is one of the major zoonotic problems that exist worldwide. Brucellosis is clinically characterized by metritis, mastitis, repeat breeding, abortion in the last trimester of pregnancy, retention of placenta and reduced milk production in the female whereas epididymitis, orchitis and sterility in male. In humans can be highly variable, ranging from nonspecific, flu-like symptoms to undulant fever, arthritis, orchitis and epididymitis.

Methods: A total of 567 bovine serum samples was taken from four districts of Brij region of UP. All the samples were processed to detection of prevalence of brucellosis by RBPT, STAT ELISA and confirmation of genes *bcsp31*, *16SrRNA, omp2* and *IS711* by PCR.

Result: The prevalence of brucellosis was found to be 07.93% (31/391), 08.69% (34/391) and 10.74% (42/391) shows positive by RBPT, STAT and I- ELISA respectively. In buffalo Out of 176 tested serum sample the seroprevalence was found to be 09.66% (17/176), 10.79% (19/176) and 12.5% (22/176) positive by RBPT, STAT and I- ELISA respectively. Out of 567 samples 18 were positive for *Brucella* genus specific gene. The higher prevalence of the disease in this region increases the risk of zoonotic transmission and it implies a serious threat to the human population as well as the huge impact on economy due to loss of productivity as well as loss of livestock population.

Key words: Bovine serum, I- ELISA, PCR, RBPT, STAT.

INTRODUCTION

Brucellosis is one of the world's major zoonotic problems that exist worldwide and is more or less endemic in most African countries and still exists in some southern European countries. Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Geering *et al*., 1995) but it remains an uncontrolled problem in regions of high endemicity such as the Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai, 2002). Almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection (CFSPH, 2003). Brucellosis is transmitted from mother to offspring before or at the birth, through milk, by sexual contact, direct physical contact, from polluted environments and from eating spoiled raw meat mainly placenta and birth products. Brucellosis is clinically characterized by metritis, mastitis, repeat breeding, abortion in the last trimester of pregnancy, retention of placenta and reduced milk production in the female whereas epididymitis, orchitis and sterility in male (Radostits *et al.,* 2000). Brucellosis is caused by members of genus *Brucella*. These are small, non-motile, aerobic, facultative intracellular, gram-negative coccobacilli. The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Fichi, 2003). The species of *Brucella* and their major hosts are *Br. abortus* (cattle), *Br. melitensis* (goats), *Br. suis* (swine) and *Br. ovis* (sheep). *Br.abortus* also causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen (Radostits *et al.,* 2000). In humans, brucellosis is considered to be an occupational disease that mainly affects slaughter house workers, butchers and veterinarians. Symptoms in human brucellosis can be highly variable, ranging from non-specific, flu-like symptoms (acute form) to undulant fever, arthritis, orchitis and epididymitis (Hassan *et al.,* 2012). Bovine brucellosis, mainly caused by *Brucella abortus*, is an important zoonotic disease leading to several public health and economic problems in endemic areas.

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MATERIALS AND METHODS

Source and place of samples collection

The present study was conducted in four districts *viz.*, Mathura, Agra, Hathras and Kasganj from August, 2017 to April, 2019. These districts were selected because of the high numbers of smallholder dairy farmers, gaushalas and good animal husbandry practices. During the present study, about 10 ml of blood from each animal and total 567 sample (391 cattle and 176 buffalo serum sample) were collected in aseptic conditions using labeled sterile disposable syringes (dispovan) or vacutainers (BD, USA). All the samples were processed in the department of veterinary public health, college of veterinary science and animal husbandry, Mathura, U.P. The serum was separated after clotting of blood and transported to laboratory on ice. All the serum samples were stored at -20°C till tested.

Prevalence based on different serological tests

Rose bengal plate test (RBPT) and standard tube agglutination test (STAT) antigen were procured from Indian Veterinary Research Institute, (IVRI), Izatnagar, India. For serum I-ELISA, kit was procured from Svanova (Biotech-AB), Uppasala, Sweden. RBPT antigen is a 8% suspension of pure smooth killed cells of *Brucella abortus* strain 99 phenolised and stained with rose Bengal dye. Rose Bengal Plate Test is a single dilution serum agglutination test. It was performed on glass slides according to the method presented by (Alton *et al.,*1988). To detection of STAT in a serum samples, *Brucella abortus* plain antigen were taken which was heat killed phenolised suspension of *Brucella abortus* strain 99 and it show 50% agglutination at 1/500 final dilution of serum with Indian standard. In bovine serum samples antibody was detected by Indirect ELISA (I-ELISA) kit procured from Svanova (Biotech-AB), Uppasala, Sweden. Briefly, each of the kit contained 96 flat bottom polystyrene with *Brucella abortus* precoated antigen wells.

Molecular characterization of *Brucella* by PCR

Bacterial DNA was extracted by Thermo scientific Gene JET Whole Blood Genomic DNA purification kit. For the PCR reaction, PCR master mix solution (Genei, Bangalore) was used. DNA isolation and PCR analysis for detection of bcsp31, omp2, 16S rRNA and IS711 gene was carried out as per the method described by Baily *et al.* (1992), Klevezas *et al.* (1992), Romero *et al.* (1995) and Doust *et al.* (2007) respectively (Table 1). PCR was carried out in a final reaction volume of 25 µl containing 12.5 µl of Master mix, 3 µl of DNA template, 1 µl of each of the primers (forward and reverse) with concentration of 15 pmol each and rest of DNAse free water. For bcsp31 gene amplification an initial denaturation step at 95°C for 4 min, denaturation at 94°C for 2 min., annealing 65°C for 2 minute, extension 72°C for 1.5 min. and followed by a final extension step at72°C for 2.5 min. For omp2 gene amplification an initial denaturation step at 94°C for 4 min, denaturation at 94°C for 1 min., annealing 60°C for 1 minute, extension 72°C for 1 min. and followed by a final extension step at72°C for 3 min. For 16S rRNA gene amplification an initial denaturation step at 95°C for 5 min, denaturation at 94°C for 5 min., annealing 54°C for 1.5 minute, extension 72°C for 1.5 min. and followed by a final extension step at72°C for 6 min. For bcsp 31 gene amplification an initial denaturation step at 95°C for 4 min, denaturation at 94°C for 2 min., annealing 65°C for 2 minute, extension 72°C for 1.5 min. and followed by a final extension step at 72°C for 2.5 min. For IS711 gene amplification an initial denaturation step at 95°C for 10 min, denaturation at 94°C for 1 min., annealing 58°C for 1 minute, extension 72°C for 1 min. and followed by a final extension step at 72°C for 7 minute. For each gene 35 amplification cycles were performed. After the amplification, amplicons were separated in 1.5% gel intris-acetate EDTA (TAE) buffer at 60 volt for 80 min, stained with 0.5% ethidiumbromide solution and visualized under ultraviolet light.

RESULTS AND DISCUSSION

Out of 391 tested cattle serum sample, the seroprevalence of brucellosis was found to be 07.93% (31/391), 08.69% (34/391) and 10.74% (42/391) shows positive by RBPT, STAT and I- ELISA respectively. In buffalo Out of 176 tested serum sample the seroprevalence was found to be 09.66% (17/176), 10.79% (19/176) and 12.5% (22/176) positive by RBPT, STAT and I- ELISA respectively. The result showed that the seroprevalence of brucellosis in buffalo was significantly higher than seroprevalence of brucellosis in cattle.

The seroprevalence of brucellosis was higher in buffalo as compare to cattle (Table 2) but significantly the prevalence in bovine brucellosis not differ (p=0.541). In present study

Table 1: Details of primers used for PCR reaction for *Brucella* genes.

Gene target and size of Amplified product (bp)	Primers	Sequences (5′-3′)	Reference
bcsp31 (223 bp)	B4 (Forward)	5′-TGG-CTC-GGT-TGC-CAA-TAT-CAA-3′	Baily *et al.* (1992)
	B4 (Reverse)	5′-CGC-GCT-TGC-CTT-TCA-GGT-CTG-3′	
omp2 (193 bp)	JPF (Forward)	5′-GCG-CTC-AGG-CTG-CCG-ACG-CAA-3′	Klevezas *et al.* (1992)
	JPF (Reverse)	5′-ACC-AGC-CAT-TGC-GGT-CGG-TA-3′	
16S rRNA (905)	Forward	5′-TCG-AGC-GCC-CGC-AAG-GG-3′	Romero *et al.* (1995)
	Reverse	5′-AAC-CAT-AGT-GTC-TCC-ACT-AA-3′	
IS711 (498 bp)	IS711AB (Forward)	5′-GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC-3′	Doust *et al.* (2007)
	IS711AB (Reverse)	5′-TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT-3′	

the sero-prevalence of brucellosis in cattle and buffalo by I-ELISA was 10.74% and 12.5% respectively in serum samples. Present finding were very close to the reports of Kebede *et al.* (2008) (11%), Eshetu *et al.* (2005) (10%) and 9.7% by Aggad and Boukraa, (2006). Higher prevalence rates were also reported in cattle in Nigeria (32.2%) (Junaidu *et al.,* 2008), Berhe *et al.* (2007) (42.31%) and Kebede *et al.* (2008) (45.9%) in Ethiopia, Aggad and Boukraa (2006) (31.5%) in Algeria and Ahmad, (2009) (25.8%) in Jordan. Some lower prevalence rate was reported by Kassahun (2004) for intensive (2.5%) and extensive farms (1.7%) in Southern Ethiopia, Algeria Berhe *et al.* (2007) (3.19%) in extensive production systems (Fig 1, 2, 3).

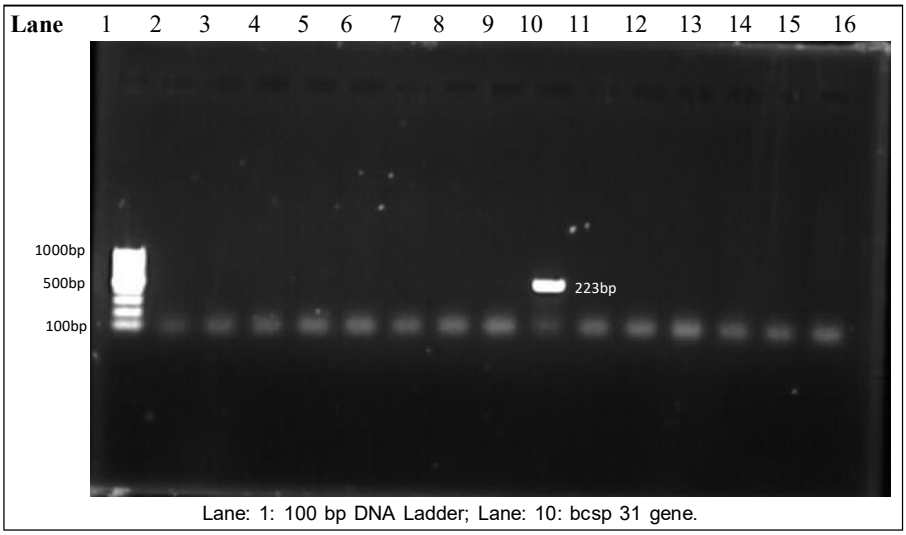
Molecular detection

In this study, primers B4/B5, F4/R2, JPF/JPR for *Brucella* genus detection and IS711ABF/ IS711ABR for *Brucella* species detection were used. These primer pairs encode fragments 223 bp, 905 bp, 193 bp and 498 bp of the genes *bcsp31*, *16SrRNA, omp2* and *IS711* genes respectively and was able to identify genus *Brucella* and species *Brucella abortus*. The aim of this study was to evaluate and compare three pairs of primers, which are broadly used for diagnosis of brucellosis cattle and buffalo serum samples. A total of 567 samples were screened by PCR to detect the presence of *Brucella* genus specific gene (bcsp31, omp2 and 16S rRNA) and *Brucella abortus* species specific IS711 genes. Out of 567 samples 18 were positive for *Brucella* genus specific gene. Out of 18 bovine serum PCR positive sample, 6 sample were positive for bcsp31, omp2 and 16S rRNA gene, 8 sample were positive for bcsp31 and omp2 gene and 4 serum samplewere positive for bcsp 31 and 16S rRNA gene. All bcsp31, omp2 and 16S rRNA gene were show positive for *Brucella* species specific IS711 (498 bp) genes. Out of four human DNA sample one positive for bcsp31, omp2 and 16S rRNA gene, 2 sample shows positive for bcsp31 and 16S rRNA gene and one sample positive for bcsp31 and omp2 gene. The *bcsp31* PCR was the most sensitive (Mukherjee *et al.,* 2005). Some modification in PCR protocol the sensitivity of the detection can also be affected (Romero *et al.,* 1995; Leal-Klevezas *et al.,* 1995). Variation in the omp2 gene sequence has been used as a basis for typing strains (Bardenstein *et al.,* 2002; Ferrao *et al.,* 2006). Some other researcher also developed PCR based assays for the identification of the genus *Brucella* from animal milk, blood, serum, tissue and from human blood and serum, these employ the gene encoding the 31 kDa Brucella cell surface salt extractable protein (BCSP), omp2, 16S rRNA, IS711 and other gene targets (Da Costa *et al.,* 1996; Rijpens *et al.,* 1996; Bricker, 2002; Morata *et al.,* 2003; Mukherjee *et al.,* 2005; Leary *et al.,* 2006). Finally it cannot be ruled out that the bcsp31 gene sequence is better conserved than the omp2 and 16S rRNA gene sequence in the genus *Brucella*. The better molecular diagnostic approach for screening of field animals and for increased sensitivity and higher specificity, more than one marker-based PCR could be use.

Table 2: Species wise seropositivity of bovine brucellosis (cattle and buffalo) detected by RBPT, STAT and I-ELISA.

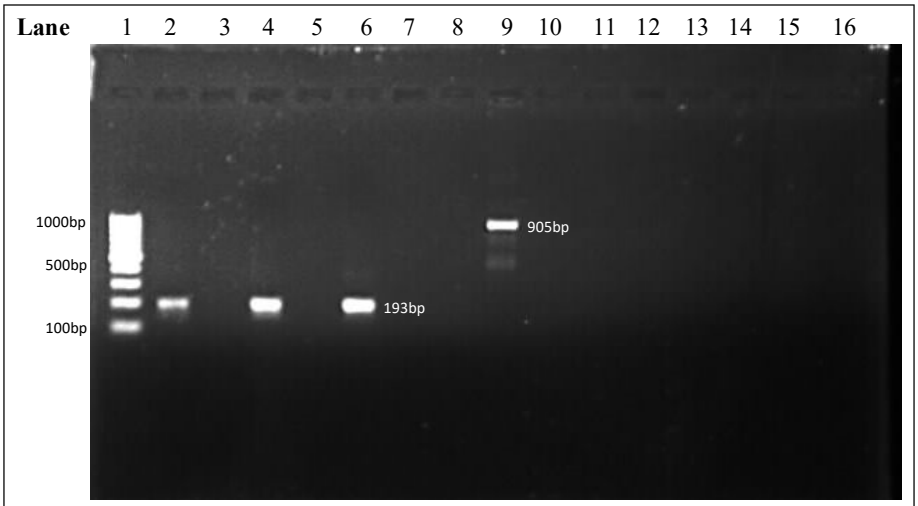
Species	No. of serum sample	No. of samples positive by RBPT	Per cent positivity by RBPT	No. of samples positive by STAT	Per cent positivity by STAT	No. of samples positive by I-ELISA	Per cent positivity by I-ELISA	p value
Cattle	391	31	07.93%	34	08.69%	42	10.74%	0.541
Buffalo	176	17	09.66%	19	10.79%	22	12.5%	
Total	567	48	08.46%	53	09.35%	64	11.28%	

p>0.05 at 5% level of significance.



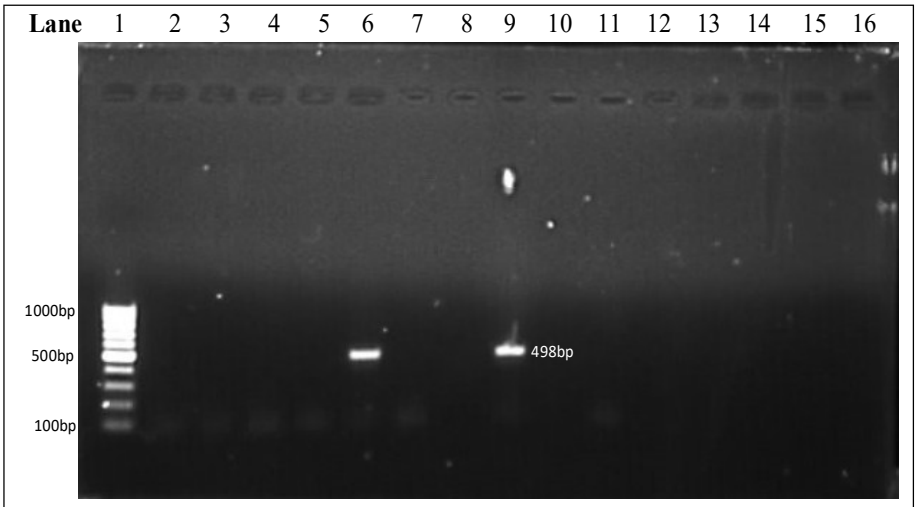
Lane: 1: 100 bp DNA Ladder; Lane: 10: bcsp 31 gene.

Fig 1: Agarose gel showing PCR amplified product for *Brucella* genus specific gene isolated from bovine serum sample.



Lane 1: 100 bp DNA Ladder; Lane 9: 16S rRNA gene; Lane 2, 4, 6: omp2 gene.

Fig 2: Agarose gel showing PCR amplified product for *Brucella* genus specific genes isolated from bovine serum sample.



Lane 1: 100 bp DNA Ladder; Lane 6 and 9: IS711 gene.

Fig 3: Agarose gel showing PCR amplified product for *Brucella* species specific genes isolates from bovine serum sample.

CONCLUSION

Out of 567 bovine serum sample, the prevalence of brucellosis in 391 cattle was found to be 07.93% (31/391), 08.69% (34/391) and 10.74% (42/391) shows positive by RBPT, STAT and I- ELISA respectively and in 176 buffalo tested serum sample the seroprevalence was found to be 09.66% (17/176), 10.79% (19/176) and 12.5% (22/176) positive by RBPT, STAT and I- ELISA respectively. 18 samples were positive in molecular detection by PCR.The higher prevalence of the disease in this region increases the risk of zoonotic transmission and it implies a serious threat to the human population as well as the huge impact on economy due to loss of productivity as well asloss of livestock population.

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