



Original Research Article

Seroprevalence and Molecular Characterization of *Brucella spp.* in Buffalo from North Gujarat, India

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ABSTRACT

Keywords

RBPT,
ELISA
and
PCR

Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals. Abortion, placentitis, epididymitis and orchitis are the most common clinical manifestations in animals. Of 118 sera of buffalo screened, 48 (40.67 %), 11 (9.32 %), 19 (16.10 %) and 11 (9.32 %) detected positive by RBPT, Lateral flow assay, Genomix i-ELISA and i-ELISA and NIVEDI, respectively. Out of 33 samples screened 07 samples (placenta-1, fetal-1, stomach content-1, fetal liver-1, spleen-1, fetal lung-1, fetal heart blood-1 and cotyledon-1,) yielded *Brucella* in genus specific PCR. Species specific PCR based on IS711 was performed of same isolates for the confirmation of *Brucella* species and samples which were detected positive, all these samples revealed as *Br. abortus*.

Introduction

Brucellosis is an important reproductive disease of livestock and is considered a reemerging infectious disease in many areas of the world. According to the World Organization for Animal Health (OIE, 2004), bovine brucellosis is a reportable zoonoses and is of considerable socio-economic concern. It is of major importance in the international trade of animals and animal products. Because of public health importance and international trade implications, all member states of the OIE have an obligation of reporting. The losses is inflict in terms of mortality, morbidity, loss of production *etc.*

In *Brucella* currently there are eight known species in terrestrial animals and two in marine animals. The species in terrestrial animals include: *B. abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. canis*, *B. ovis*, *B. microti* and *B. inopinata*. The most common domestic animals affected by brucellosis are Cattle, Buffaloes, Goats, Sheep and Pigs (Bamaiyi Pwaveno *et al.*, 2012). In literature search reveals that various workers have reported the existence of Brucellosis in various parts of Gujarat, however there exist a paucity of information on epidemiology of brucellosis in buffaloes. So, present study was aimed with objective with

seroprevalence and isolation of *Brucella* from North Gujarat.

Materials and Methods

Samples collection from buffalo

Total 118 serum samples and 33 clinical samples (placenta, fetal, stomach content, fetal liver, spleen, fetal lung, fetal heart blood and cotyledon) were collected from rural areas and organized farms belonging to two districts viz., Banaskantha and Sabarkantha. The serum samples were heat inactivated at 56°C for 30 min. and merthiolate (1 : 10,000) was added in all vials as a preservative and sera were stored at -20°C till further use.

Seroprevalence

Rose Bengal Plate Test (RBPT)

The RBPT antigen was procured from the Institute of Animal Health and Veterinary Biologicals (IAH and VB), Hebbal, Bangalore, Karnataka-560 024 and the test was performed as per the protocol outlined in the user manual given by Institute.

Lateral flow Immunochromatographic Assay

Antibody detection rapid test kit based on lateral flow Immunochromatographic Assay was made available by courtesy of Genomix Molecular Diagnostics Pvt. Ltd., and the test was performed as per the protocol outlined in the user manual given by manufacturing institute.

Indirect-enzyme linked Immunosorbent Assay

Brucella Antibody Test Kit, ELISA was made available from National Institute of

Veterinary Epidemiology and Disease Informatics (NIVEDI) formerly Animal Disease Monitoring and Surveillance (ADMAS), Bangalore. The test was performed as per the protocol outlined in the user manual.

Genomix *Brucella* Indirect ELISA

Brucella Antibody Test Kit, ELISA was made available by courtesy of Genomix Molecular Diagnostics Pvt. Ltd., Hyderabad was used in the present study. The test was performed as per the protocol outlined in the user manual and the results obtained were also compared with that of NIVEDI kits.

Molecular characterization of *Brucella* isolates:-

DNA Extraction: - DNA extraction from clinical samples was performed by using QIAmp DNA Mini Kit-Qiagen.

Quality checking of extracted DNA: - The quality and purity of DNA were checked by Agarose Gel Electrophoresis and by Picodrop method (Picodrop, U.K.). Genus specific PCR for detection of *Brucella* was performed by using primer and protocol given in table 1.

Procedure

25 µl PCR reaction mixture was made as per table 2.

Visualization of PCR products by agarose gel electrophoresis

Procedure

To confirm the targeted PCR amplification, 5 µl of the PCR products from each tube was mixed with 1 µl of 6X gel loading buffer and electrophoresed along with DNA

molecular weight marker (Gene Ruler, MBI Fermentas) on 2.0 per cent agarose gel containing ethidium bromide (@ 0.5 µg/ml) at constant 80V for 30 min in 0.5 X TBE buffer. The amplified product was visualised as a single compact band of expected size under UV light and documented by gel documentation system (Mini BiS BioImaging System).

Species specific PCR was performed by using primer and following the condition given in table 3. Visualization of PCR products of Species specific PCR was same as Genus specific PCR

Result and Discussion

Seroprevalence

From 118 sera samples of buffalo tested, 48 (40.67 %), 11 (9.32 %), 19 (16.10 %) and 11 (9.32 %) were found positive for antibody by RBPT, Lateral flow assay, i-ELISA (Genomix) and i-ELISA (NIVEDI, Bangaluru) during present study (Fig. 1 & 2). The present findings corroborates the reports of Tyshete (2001) who reported over all seroprevalence of 14.16 per cent in buffaloes from North Gujarat by i-ELISA. Sharma and Saini (1995) showed 14.61 per cent positive in buffalo in Punjab. However, in contrast to the present study higher rate of seroprevalence of 28.00 per cent in buffaloes in Bangladesh have been reported by Ahmed *et al.* (2010) by ELISA and Mohamoud (1991) reported lower seroprevalence in buffaloes 3.09 per cent and Isloor *et al.* (1998), 1.8 per cent in buffaloes.

Detection of *Brucella* by genus and species specific PCR

A total of 33 clinical samples *viz.*, vaginal swab; vaginal discharge, placenta, milk fetal

stomach content, fetal liver, spleen, fetal lung, fetal heart blood, serum, cotyledon, Were screened for detection of *Brucella* using bcs31 Genus specific PCR and IS711 species specific PCR.

Of these, placenta-1, fetal-1, stomach content-1, fetal liver-1, spleen-1, fetal lung-1, fetal heart blood-1 and cotyledon-1, samples found positive in Genus specific PCR yielding 223bp when electrophoresed though 2 per cent agarose gel (Fig. 3). And same samples found positive in species specific PCR yielding 495 bp when electrophoresed through 2 per cent agarose gel. Same study was also performed by various workers in different place either from blood or vaginal secretions independently for direct detection of *Brucella* by PCR (Morata *et al.*, 2001, Zerva *et al.*, 2001; Varasada, 2003).

PCR detected the DNA of specific *Brucella* species supposed to be present in animals which are actually suffering from brucellosis. Further, it also detected the *Brucella* at species level, so that treatment and disease eradication at species level can be taken up at the earliest. This is not possible in case of serology based diagnosis of brucellosis wherein serological tests detect merely the presence of antibodies rather the presence of infection. Therefore, PCR will not only overcome all the drawbacks of serological tests but is also helpful in identifying the presence of *Brucella* infection without isolation. Therefore, PCR assay can be employed as an alternative technique in such cases, although isolation of causative agent has been considered as gold standard for diagnosis of brucellosis.

Table.1 Genus specific primers for PCR

Genus specific primers :					
Sr. No	Primer	Forward/Reverse	Sequence (5'-3')	Product size (bp)	Reference
1.	B4	Forward	TGG CTC GGT TGC CAA TAT CAA	223 bp	<i>Bailly et al. (1995)</i>
2.	B5	Reverse	CGC GCT TGC CTT TCA GGT CTG		

Table.2 PCR reaction mixture

Quantity and concentration of various components used in PCR

Sr. No.	Components	Colony PCR	
1.	PCR Master Mix (2X)	12.5 µl	
2.	Forward Primer (10 pmol/µl)	1 µl	
3.	Reverse Primer (10 pmol/µl)	1 µl	
4.	Template DNA	2 µl	
5.	Nuclease free water	8.5 µl	
Steps and conditions of thermal cycling for different primer pairs in PCR for B4/B5			
Steps	Temperature	Duration	Number of cycle
Initial Denaturation	93°C	5 min.	1 cycles
Denaturation	90°C	1 min.	35 cycles
Annealing	64°C	30 sec.	
Extension	72°C	1 min.	
Final extension	72°C	10 min.	1 cycles

Table.3 Species specific PCR was performed by using following primer and condition

Species specific primers :					
Sr. No	Primer	Forward/Reverse	Sequence (5'-3')	Product size (bp)	Reference
1.	IS711 (<i>B. abortus</i>)	Forward	GACGAACGGAATTTTCCAATCCC	498 bp	Bricker and Halling (1994)
2.	IS711 (<i>B. abortus</i>)	Reverse	TGCCGATCACTTAAGGGCCTTCAT		
Steps and conditions of thermal cycling for different primer pairs in PCR for <i>B. abortus</i> (IS711)					
Steps	Temperature		Duration	Number of cycle	
Initial Denaturation	95°C		2 min.	1 cycles	
Denaturation	95°C		1.15 min.	35 cycles	
Annealing	55.5°C		2 min.		
Extension	72°C		2 min.		
Final extension	72°C		5 min.	1 cycles	

Figure: 1. Rose Bengal Plate Test (RBPT)

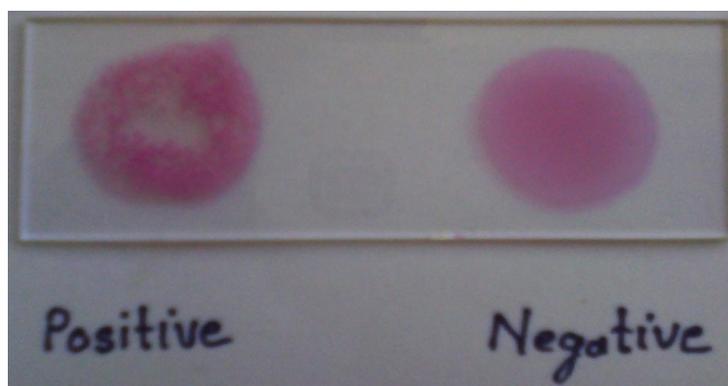
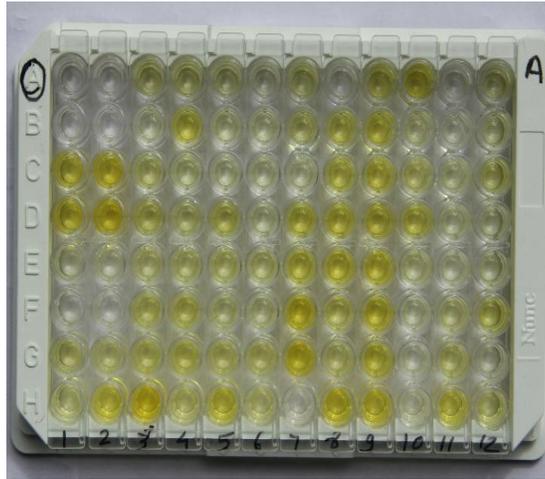


Figure.2 i-ELISA for detection of *Brucella* antibodies (Genomix kit)

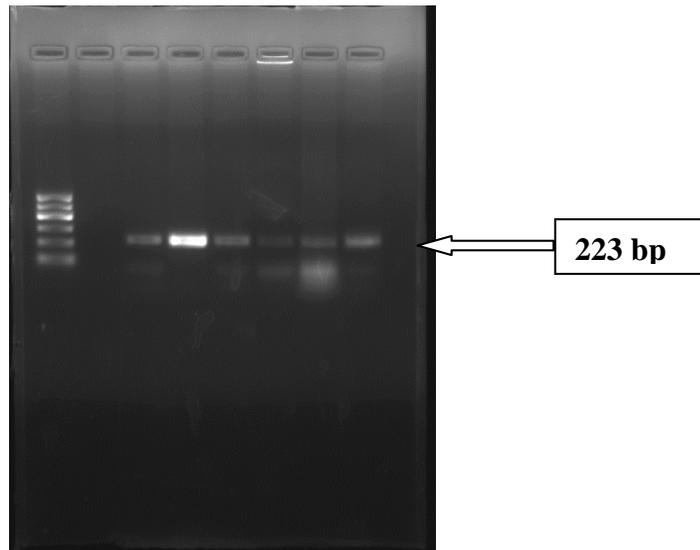


Wells A1, B1, A2, B2: Negative control

Wells C1, D1, C2, D2: Moderately positive control

Wells E1, F1, E2, F2, G1, G2, H1, H2 , A3, B3, C3, D3, C5, D5 etc. field sera

Figure.3 Agarose Gel electrophoresis of 223bp PCR products with bcs31 primers



1- Ladder

2-NTC

3- sample (positive)

4- sample (positive) (Culture DNA)

5-sample (positive)

6- sample (positive)

7- sample (positive)

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