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A comparative study of serology and PCR for the diagnosis of brucellosis in goats

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ABSTRACT

Brucellosis is an economically important infectious disease of livestock causing abortions, infertility, delayed oestrus, interrupted lactation, increased condemnation and loss of milk production besides its zoonotic nature. The present study was conducted to compare serological assays and polymerase chain reaction (PCR) for the diagnosis of caprine brucellosis. A total of 301 whole blood samples to extract DNA for PCR and serology were collected from goats maintained at various organized herds, panjarapoles, slaughter house, local meat markets, etc. in South Gujarat region of India. Out of 301 serum samples tested, 7 samples (2.33%) were positive by all the three serological tests, viz. rose bengal plate test (RBPT), indirect ELISA (iELISA) and immunochromatographic assay (ICA). Among 301 DNA samples, genus specific PCR detected DNA of *Brucella* spp. in 11 samples by targeting *BCSP 31* and *IS-711* genes to get 223 bp and 350 bp PCR products on agarose gel electrophoresis. None of the seven serologically positive samples showed *Brucella* genus-specific DNA amplification by PCR and similarly all PCR positive samples were negative on serology.

Key words: Brucellosis, ELISA, Goats, ICA, PCR, RBPT

The first outbreak of brucellosis among animals was reported in goats in Mediterranean Island Malta by Zammit (1905) who also established its zoonotic nature by isolating the organism from goat milk. Though its distribution is worldwide, it is more common in countries having poor animal and public health programmes (Capasso 2002). Caprine brucellosis caused by *B. melitensis* is widely prevalent in our country and is a threat to human beings due to its zoonotic nature. Abortion during late pregnancy is the most obvious sign in goats and sheep. Although not all infected goats abort but they do shed *Brucella* into the environment. Among pregnant ruminants, over 85% of *Brucella* organisms can be found in the cotyledons, placental membranes, amniotic and allantoic fluid (Poester *et al.* 2013). Therefore, infected goats can transmit the infection to in-contact goats and animal handlers on a farm.

Apart from clinical signs, specific diagnosis is usually based on a battery of laboratory tests. Bacterial isolation, serological tests and molecular approaches are routinely used for the diagnosis of brucellosis in various species of animals. Isolation and identification of *Brucella* spp. is considered as the gold standard test. Serological tests and their modifications have been used by various workers to detect antibodies against *Brucella* spp. such as Rose Bengal plate test (RBPT), Milk Ring test (MRT) and ELISA to name a few (Trangadia and Prasad 2018a). Polymerase chain reaction (PCR) is sensitive, specific, rapid and relatively inexpensive method for detecting *Brucella* spp. in variety of samples. PCR is an important tool for the diagnosis of caprine brucellosis because a significant proportion of the infected animals show negative results in the serological tests. A number of nucleic acid sequences including 16S rRNA, 16S-23S rRNA, BCSP-31, OMP2b, OMP-31, *IS 711* and BP-26 have been targeted for *Brucella* genus-specific PCR assays (Gupta *et al.* 2014, Sonekar *et al.* 2018). Therefore, the present study attempted to compare the results of serological tests and PCR for the diagnosis of brucellosis in goats.

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MATERIALS AND METHODS

Source of the samples: During the study, a total of 301 whole blood samples for DNA extraction/PCR and serology were collected from goats maintained at various organized herds, panjarapoles, slaughter house, local meat markets, etc. in South Gujarat region of India (Table 1). Serum samples were stored at –20°C until used. All these animals were above 6 months of age and none of these animals were vaccinated against brucellosis as per history.

Rose Bengal plate test (RBPT): RBPT antigen was procured from the Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India. In brief, RBPT was performed by mixing a drop of RBPT reagent with an equal volume of serum and the test was read for agglutination within 4 min.

Indirect ELISA (iELISA): A commercial ID Screen® Brucellosis serum indirect multi-species ELISA test kit (IDVet, France) was used to screen these samples for detecting anti-brucella antibodies and the test was

Table 1. Samples collected from goats

Location	Sex	No. of whole blood samples
Slaughter house,	Male	52
Surat, Gujarat	Female	24
	Sub-total	76
Maroli meat	Male	30
market, Gujarat	Female	25
	Sub-total	55
Panjarapole, Navsari	Male	24
Gujarat	Female	13
	Sub-total	37
Panjarapole, Vapi	Male	11
Gujarat	Female	15
	Sub-total	26
Organized farm,	Male	3
Bilimora, Gujarat	Female	36
	Sub-total	39
Organized farm	Male	8
Bharuch, Gujarat	Female	60
	Sub-total	68
	Grand Total	301

performed as described by the manufacturer. The S/P % was calculated as S/P % = [(OD sample – mean OD negative control) / (mean OD positive control – mean OD negative control)] × 100. Samples with an S/P % less than or equal to 110 were classified as negative and S/P % greater than or equal to 120 were classified as positive. Those with an S/P % between 111 and 119 were classified as doubtful, which subsequently retested by ELISA to classify either as negative or positive.

*Immunochromatographic assay (ICA):*A commercial Antigen Rapid GS *Brucella* Ab chromatographic immunoassay kit (BioNote Inc., Republic of Korea) was used to screen these animals for the presence of anti-brucella antibodies. Briefly, 10 µl of serum sample was added to sample well using a capillary tube and four drops of assay diluent were added over it. The test result was interpreted at 20 min. If sample is negative, only control line (single line) appeared and both control and test lines (two lines) appeared in positive samples.

Extraction of genomic DNA from blood samples: The genomic DNA was extracted from blood using DNeasy Blood and Tissue Kit (Qiagen-cat No. 69504). The quality of the extracted DNA was assessed by submarine agarose gel electrophoresis using 0.8% agarose in 0.5× Tris-Borate and EDTA (TBE) buffer (*p*H 8.0) with ethidium bromide (Sambrook and Russell 2001). The DNA was visualized under UV transilluminator (BioScreen Instrumentation Pvt. Ltd.). The quantity and purity of extracted DNA samples were checked using Nano-Drop Spectrophotometer ND 2000c (Thermo Scientific) at 260/280 nm wavelength. Extracted DNA samples were stored at –20°C till further processing.

Polymerase chain reaction (PCR): DNA samples were used as template for *Brucella* genus-specific multiplex PCR to amplify the fragment of 223 bp and 350 bp targeting BCSP-31 and IS-711 gene, respectively (Baily *et al.* 1992, Henault *et al.* 2000). Details of primers are listed in Table 2. The PCR was standardized and performed in 25 µl reaction mixture containing 12.5 µl of 2× *Taq*PCR Master Mix (Qiagen, Germany) having *Taq* DNA Polymerase, PCR buffer containing 1.5 mM $MgCl_2$ and dNTPs, forward and reverse primers (0.5 µl each of BCSP-31 and IS-711) containing 10 pmol/µl, 2 µl of DNA template and 8.5 µl of nuclease free water. The PCR amplifications were performed in ABiVeriti (Applied Biosystems) with an initial denaturation at 95°C for 3 min followed by 35 cycles each of denaturation for 45 sec at 95°C, annealing at 60°C for 45 sec, extension at 72°C for 2 min and a cycle of final extension at 72°C for 10 min. The PCR products were mixed with 1 ml of loading dye and were electrophoresed through 2% agarose gel pre-mixed with 1% ethidium bromide (5 µg/100 ml) in 0.5× TBE at 80 V for 30 min. Three microlitres of 100 bp DNA marker (Gelpilot, Qiagen, Germany) was run simultaneously. The amplified products were visualized under UV transilluminator in a gel documentation system (BioScreen Instrumentation Pvt. Ltd.). Samples showing amplified products of 223 and 350 bp size for BCSP-31 and IS-711 gene, respectively were confirmed as *Brucella* spp.

Table 2. Primers used for detection of genus of *Brucella* organisms

	Primer sequence (5′–3′)	Product size
BCSP-31 (F)	TGGCTCGGTTGCCAATATCAA	223 bp
BCSP-31 (R)	CGCGCTTGCCTTTCAGGTCTG	
IS-711 (F)	CTGGCTGATACGCCGGACTTTGAA	350 bp
IS-711 (R)	GGAACGTGTTGGATTGACCTTGAT	

BCSP31, *Brucella* cell surface extractable protein gene 31; *IS711*, Insertion Sequence 711; F, Forward primer; R, Reverse primer.

RESULTS AND DISCUSSION

Conventionally, serological tests are used world over for screening of cattle for brucellosis. But serological tests including ELISA sometimes lead to cross reaction of *Brucella spp.* with other bacteria especially *Yersinia enterocolitica* O:9 and may result in a false positive reaction for brucellosis (See *et al.* 2012). Whereas, serological tests are not very specific in areas where the disease is highly endemic (Gupta *et al.* 2006a,b). Hence, serological tests along with PCR may be used to arrive at a conclusive diagnosis. The present study compared the results of serology and PCR based on 301 sera and whole blood samples collected from six different locations in South Gujarat for the diagnosis of caprine brucellosis. Observations on serological tests and PCR are depicted in Table 3.

Out of 301 sera samples tested, 7 samples (2.33%) comprising 4 male and 3 female animals were positive by all the three serological tests, viz. RBPT, iELISA and ICA. However, PCR detected DNA of *Brucella* spp. in 11 samples

Table 3. Comparison of test results by serology and PCR

Location	Animal ID (sex)	Serology			Genus specific
		RBPT	iELISA	ICA	PCR
Slaughter house, Surat, Gujarat	S12 (M)	Neg	Neg	Neg	Pos
	S15 (M)	Neg	Neg	Neg	Pos
	S18 (M)	Neg	Neg	Neg	Pos
	S21 (M)	Neg	Neg	Neg	Pos
	S29 (M)	Neg	Neg	Neg	Pos
	S36 (F)	Neg	Neg	Neg	Pos
Maroli meat market, Gujarat	M5 (M)	Pos	Pos	Pos	Neg
	M11 (M)	Pos	Pos	Pos	Neg
	M21 (M)	Pos	Pos	Pos	Neg
Panjarapole, Navsari, Gujarat	K12 (M)	Pos	Pos	Pos	Neg
Panjarapole, Vapi, Gujarat	R12 (M)	Neg	Neg	Neg	Pos
	R20 (M)	Neg	Neg	Neg	Pos
Organized farm, Bilimora, Gujarat	B16 (F)	Neg	Neg	Neg	Pos
	B27 (F)	Neg	Neg	Neg	Pos
Organized farm, Bharuch, Gujarat	V10 (F)	Pos	Pos	Pos	Neg
	V42 (F)	Neg	Neg	Neg	Pos
	V47 (F)	Pos	Pos	Pos	Neg
	V51 (F)	Pos	Pos	Pos	Neg

RBPT, Rose Bengal Plate Test; iELISA, Indirect Enzyme-linked Immunosorbent Assay; ICA, Immunochromatographic Assay; M, Male; F, Female; Neg, Negative; Pos, Positive. *Brucella* Genus specific PCR for BCSP-31 and IS-711 sequences.

comprising 7 male and 4 female animals by targeting *BCSP 31* and *IS 711* gene to get 223 bp and 350 bp PCR products on agarose gel electrophoresis (Supplementary Fig. 1). The remaining 283 samples were negative by serology and PCR. In the present study, all the seven serologically positive samples did not show *Brucella* genus-specific DNA amplification by PCR and similarly all PCR positive samples were serologically negative (Table 3).

Comparable rate of seroprevalence was reported from Uttar Pradesh (2.90%) by Sharma *et al.* (1984) and Gujarat (3.3%) by Trangadia and Prasad (2018b). Comparatively higher rate of incidence (8.80 to 59.26%) was noted in Gujarat (Sutariya *et al.*2014, Sadhu *et al.* 2015, Patel *et al.* 2016), 8.27% in goats of Rajasthan, Gujarat and Karnataka (Shome *et al.*2006) and 22.58% in Nagpur region of Maharashtra (Raju *et al.* 2004) by RBPT and other serological tests. However, lower rate of seroprevalence of 1.65% was recorded by Sonawane *et al.* (2011) in Rajasthan and 1.75% in Punjab (Sharma and Saini 1995). The variation in seroprevalence rate in different states may be due to husbandry and management practices followed at individual farms (Pathak *et al.* 2016).

Genus-specific PCR targeting *BCSP-31* and *IS-711* genes and species-specific PCR are used for the diagnosis of brucellosis from various clinical specimens (Vivekananda *et al.* 2012, Kurkure *et al.* 2016, Pathak *et al.* 2016, Sonekar *et al.* 2016). Genus-specific PCR detected DNA of *Brucella* spp. in 11 samples by targeting *BCSP-31* and *IS-711* gene to get 223 bp and 350 bp PCR products, respectively. In the present study, all the seven serologically positive samples did not show *Brucella* genus-specific DNA amplification by PCR and similarly, 11 PCR positive samples were serologically negative as also reported by Gupta *et al.* (2014).

Ali *et al.* (2015) opined that species-specific PCR assays have a lower analytical sensitivity than do genus-specific PCRs. The PCR results have always been variable when compared with serology and bacteriology. The stage of infection may also influence the number and location of bacteria. At the time of sampling, the stages of infection in animals used for the present study was not known due to absence of complete history of these cases. The presence of anti-brucella antibodies indicates exposure to the bacteria but does not necessarily mean that the animals have a current or active infection. The possible explanation for serological positive and PCR negative animals might be due to higher serum antibody titers at the time of sample collection and absence of organisms as animals shed organisms in blood at early stage of infection. Secondly the presence of large amounts of genomic DNA might have inhibitory effects on PCR assays. Thirdly, the method of bacterial DNA extraction from blood samples is crucial (O'Leary *et al.* 2006).

However, the seropositive animals with negative PCR in the present study might be representing the animals at different stages of infection when the bacterial DNA is cleared from the blood stream as suggested by Gwida *et al.* (2016). The present study indicated the presence of antibodies against brucellosis in goats irrespective of clinical signs and abortion history.

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