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 \textit{Brucella} spp. in 11 samples by targeting \textit{BCSP 31} and \textit{IS-711} genes to get 223 bp and 350 bp PCR products on agarose gel electrophoresis. None of the seven serologically positive samples showed \textit{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 \textit{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 \textit{Brucella} into the environment. Among pregnant ruminants, over 85% of \textit{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 \textit{Brucella} spp. is considered as the gold standard test. Serological tests and their modifications have been used by various workers to detect antibodies against \textit{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 \textit{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 \textit{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.

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.

\textit{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.

\textit{Indirect ELISA (iELISA):} A commercial ID Screen® Brucellosis serum indirect multi-species ELISA test kit (ID Vet, France) was used to screen these samples for detecting anti-brucella antibodies and the test was...
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 120 were classified as negative, S/P % between 111 and 119 were classified as doubtful, or equal to 120 were classified as positive. Those with an S/P % greater than 120 were classified as positive and S/P % less than or equal to 110 were classified as negative.

**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 after 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 EDTA (TBE) buffer (pH 8.0) with ethidium bromide (5 µg/ml) in 0.5x 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.*

**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 2x TaqPCR Master Mix (Qiagen, Germany) having Taq DNA Polymerase, PCR buffer containing 1.5 mM MgCl₂, 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 0.5x 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.*

**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

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**Table 1. Samples collected from goats**

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

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

| Primer sequence (5'–3') | Product size |
|--------------------------|--------------|
| BCSP-31 (F) TGGCTCCTGGTCCAATATCAA | 223 bp |
| BCSP-31 (R) CGGCTTGGTCTCACGGTCTG | |
| IS-711 (F) CTGGCTGATAAGCGCGGACTTTGAA | 350 bp |
| IS-711 (R) GGAACGTGTTGGATTGACCTTGAT | |

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

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

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 |
|                              | 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.
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