SEROPREVALENCE AND MOLECULAR CHARACTERIZATION OF BRUCELLA SPP. IN CATTLE FROM NORTH GUJARAT

PATEL BHUMIKA, C. AND JAIN, B. K.,
M.G. SCIENCE INSTITUTE, DADASAHEB MAVLANKAR CAMPUS, NAVRANGPURA, AHMEDABAD.

Corresponding author’s e-mail: bhoomika71212@gmail.com

ABSTRACT:
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 135 sera of cattle screened, 37 (27.40 %), 18 (13.33 %), 17 (12.59 %) and 20 (14.81 %) detected positive by RBPT, Lateral flow assay, Genomix i-ELISA and i-ELISA and NIVEDI, respectively. Out of 23 samples screened 03 samples (vaginal swab-2 and placenta-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.

KEY WORDS: RBPT, ELISA, PCR.

INTRODUCTION:
The health of both animal and human population is pivotal in view of the economic development, stability and prosperity. The burden of infectious diseases affects health and reproductivity of livestock, thereby greatly reducing its value and opportunities for trade. The disease is caused by organisms of the genus Brucella that includes six closely related species. However, on the basis of their high DNA sequence similarity, Verger et. al., (1985). Conventional diagnosis of brucellosis is based on clinical observations and is complemented by serology and microbiological culture tests. In literature search reveals that various workers have reported the existence of Brucellosis in various parts of
Gujarat. So, present study was aimed with objective with seroepidemiology and isolation of brucella in cattle from North Gujarat.

**MATERIALS AND METHODS:**

**SAMPLES COLLECTION FROM CATTLE**

Total 135 serum samples and 23 clinical samples (Vaginal swab, Placenta, Milk and Serum) 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.

**SEROPRAVELENCE:**

1. **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.

2. **LATERAL FLOW IMMUNOCROMATOGRAPHIC ASSAY**
   Antibody detection rapid test kit based on lateral flow Immunochromatographic Assay was made available by courtesy of Genomix Molecular DiagnosticsPvt. Ltd., and the test was performed as per the protocol outlined in the user manual given by manufacturing institute.

3. **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.

4. **GENOMIX BRUCELLA INDIRECT ELISA**
   Brucella Antibody Test Kit, ELISA was made available by courtesy of Genomix Molecular DiagnosticsPvt. 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 NIVIDI kits.

5. **MOLECULAR CHARACTERIZATION OF BRUCELLA ISOLATES:**
   **DNA Extraction:** - DNA extraction from clinical samples was performed by using QIAmp DNA Mini
   **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 Brucellawas performed by using following primer and protocol.

<table>
<thead>
<tr>
<th>Genus specific primers :</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr. No</td>
<td>Primer</td>
</tr>
<tr>
<td>1</td>
<td>B4</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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PROCEDURE
25 μl PCR reaction mixture was made as per below table.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Components</th>
<th>Colony PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PCR Master Mix (2X)</td>
<td>12.5 μl</td>
</tr>
<tr>
<td>2.</td>
<td>Forward Primer (10 pmol/μl)</td>
<td>1 μl</td>
</tr>
<tr>
<td>3.</td>
<td>Reverse Primer (10 pmol/μl)</td>
<td>1 μl</td>
</tr>
<tr>
<td>4.</td>
<td>Template DNA</td>
<td>2 μl</td>
</tr>
<tr>
<td>5.</td>
<td>Nuclease free water</td>
<td>8.5 μl</td>
</tr>
</tbody>
</table>

**Table**: Quantity and concentration of various components used in PCR

**Steps and conditions of thermal cycling for different primer pairs in PCR for B4/B5**

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Duration</th>
<th>Number of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>93°C</td>
<td>5 min.</td>
<td>1 cycles</td>
</tr>
<tr>
<td>Denaturation</td>
<td>90°C</td>
<td>1 min.</td>
<td>35 cycles</td>
</tr>
<tr>
<td>Annealing</td>
<td>64°C</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>1 min.</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>10 min.</td>
<td>1 cycles</td>
</tr>
</tbody>
</table>

**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 BiSBioImaging System).
SPECIES SPECIFIC PCR WAS PERFORMED BY USING FOLLOWING PRIMER AND CONDITION.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Primer (B. abortus)</th>
<th>Forward/Reverse</th>
<th>Sequence (5’-3’&lt;sup&gt;*&lt;/sup&gt;)</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>IS711</td>
<td>Forward</td>
<td>GACGAACGGAATTTTTCCAATCCC</td>
<td>498</td>
<td>Bricker and Halling (1994)</td>
</tr>
<tr>
<td>2.</td>
<td>IS711</td>
<td>Reverse</td>
<td>TGCCGATCCTTAAGGGCCTTCAT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Steps and conditions of thermal cycling for different primer pairs in PCR for *B. abortus* (IS711):

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Duration</th>
<th>Number of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95°C</td>
<td>2 min.</td>
<td>1 cycles</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>1.15 min.</td>
<td>35 cycles</td>
</tr>
<tr>
<td>Annealing</td>
<td>55.5°C</td>
<td>2 min.</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>2 min.</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>5 min.</td>
<td>1 cycles</td>
</tr>
</tbody>
</table>

Visualization of PCR products of Species specific PCR was same as Genus specific PCR.

**RESULTS AND DISCUSSION:**

**Seroprevalence**
From 135 sera samples of cattle tested, 37 (27.40 %), 18 (13.33 %), 17 (12.59 %) and 20 (14.81 %) were found positive for antibody by RBPT, Lateral flow assay, i-ELISA (Genomix) and i-ELISA (NIVEDI, Bangaluru) during present study (Fig. 1 and 2). In contrast to present results the Kala (2009) reported that the prevalence of brucellosis in cattle was 40.88 per cent. While in ELISA similar result was revealed by Patel (2007) overall seropositivity of 29.00 per cent with prevalence of 38.29 per cent in cattle per cent in Gujarat by using i-ELISA. Contrast result was by Mahato et al. (2004) 47.76 per cent positivity for *Brucella* antibodies in cows by ELISA.

**Detection of Brucella by genus and species specific PCR**
A total of 23 clinical samples viz., Vaginal swab, Placenta, Milk and Serum were screened for detection of Brucella using bsp31Genus specific PCR and IS711 species specific PCR.

Of these, 2 vaginal sawb and 1 Placenta samples found positive in Genus specific PCR yielding 223bp when electrophoresed though 2 per cent agarose gel and same samples found positive in species specific PCR yielding 498 bp when electrophoresed through 2 per cent agarose gel (Fig. 3).
The findings are in agreement with Maria et. al., (1997); Morata et. al., (2001); Navarro et. al., (2002); Stella et. al.,(2007). By considering the results of the present study and earlier reports of other workers. This is an important issue to evaluate the suitability of clinical material to be selected for PCR. Keeping this in view, in the present work, clinical material such as blood, serum and vaginal samples collected from the same animal were studied by PCR. The results of the present study showed that the vaginal swab/secretion were the most suitable clinical specimen for PCR based detection of Brucella in livestock.

The present study was undertaken to find out the prevalence of Brucella from cattle in North Gujarat which will helpful to create the Seroepidemilogical Maps in this region. Isolation and molecular characterization by PCR of Brucella isolated helpful to make effective vaccine preparation for control of Brucella infection in cattle of North Gujarat.

ACKNOWLEDGEMENT:

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REFERENCES:


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**Fig. 1.** Lateral Flow assay for detection of Brucella antibodies

C- Positive control

T- Test serum (negative –A)

T-Test serum (Positive-B)
Figure: 2. i-ELISA for detection of *Brucella antibodies* (*NIVEDI kit*)
Wells A1, B1, A2, B2: Negative control
Wells C1, D1, C2, D2: Moderately positive control
Wells E1, F1, E2, F2: Strong positive control
Wells A3, B3, C3, D3, C5, D5 etc. field sera indicate positive reaction

Figure: 3. Agarose Gel electrophoresis of 495 bpPCR product with primer IS711
1- Ladder
2- NTC
3- Sample (positive)
4- Sample (Negative)
5- Sample (Negative)