Comparative Efficacy of Serological Tests for Detection of Brucella Antibodies in Sheep and Goats


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ABSTRACT

Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals. In view of the considerable problems related to direct diagnosis of brucellosis in animals, the present study envisaged the appraisal of seroepidemiology of brucellosis in sheep and goats by detection of brucella specific antibodies, comparison of two serological tests, viz., i-ELISA (Indirect Enzyme Linked Immunosorbent Assay) and RBPT expand for detection of Brucella-specific antibodies. Out of 1012 sheep and goat sera screened, 88 (8.70%) and 75 (7.41%) were detected positive by RBPT and i-ELISA, respectively. Species-wise seroprevalence was detected 12.26% and 10.97% in sheep and 5.67% and 4.39% in goats by RBPT and i-ELISA, respectively. During present investigation, RBPT detected more number of samples positive for brucella antibodies. However, compared to i-ELISA, overall sensitivity and specificity of RBPT were 80.00% and 97.01%, respectively. Species-wise sensitivity of RBPT found was 82.35% in sheep and 75.00% in goat, whereas specificity was 96.38% in sheep and 96.41% in goats.

Keywords: Brucellosis, B. melitensis, RBPT, i-ELISA, seroprevalance

Brucellosis due to Brucella melitensis is widespread in India and is considered to be the major cause of abortion in small ruminants incurring severe economic loss. Free grazing and movement with frequent mixing of flocks of sheep and goats also contribute to the wide distribution of brucellosis in these animals. In Sheep and Goat, the average economic annual loss due to brucellosis per animal was found to be ₹ 1180 and ₹ 2121.82, respectively. (Sulima and Venkataraman et al., 2010). Appreciating the economic losses of brucellosis, Department of Biotechnology (DBT), New Delhi has initiated a nationwide network project on brucellosis. Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar is one of the co-opting centre. The Brucellosis diagnosis (and surveillance) almost entirely rely on serological tests, e.g., Rose Bengal Plate Test (RBPT), Standard Agglutination Test (STAT), indirect Enzyme Linked Immunosorbent Assay (i-ELISA), and Complement Fixation Test (CFT), that detect antibodies against Brucella antigens including lipopolysaccharides (LPS) and give indirect evidence of Brucella infection (Godfroid et al., 2002; Adone and Pasquali, 2013). The major drawbacks of these assays are that they are not always specific and can cross react with other gram negative bacteria like Yersinia enterocolitica, Vibrio cholerae, Campylobacter fetus, Bordetella bronchiseptica and Salmonella spp. (Corbel and Brinley-Morgan, 1984) and antibodies are not produced in the acute stage of infection (Moussa et al., 2011). So, the main aim of present study is which is the better serological test in sense of sensitivity and specificity from RBPT and i-ELISA for diagnosis of Brucellosis.

MATERIALS AND METHODS

The present work on presence or absence of brucella antibodies in serum samples collected from sheep and goats. A total of 1012 serum samples were collected...
from rural areas and organised farms belonging to five districts (Banaskantha, Patan, Navsari, Vapi and Kutchch) of Gujarat from 2014 to 2016. About 9 ml of blood was collected aseptically from the jugular vein of individual animal in a vacuette with serum clot activator (Greiner bio-one, Austria). The vacuette were kept in upright position at room temperature for about 2 hrs. The separated serum samples were collected and stored at -20ºC till further use.

**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. The test was carried out by mixing 0.03 ml of serum and 0.03 ml of *B. abortus* Rose Bengal coloured antigen on a slide and mixed thoroughly with sterile tooth picks and then the slide was rotated and observed for reaction upto four min. The results were recorded. Definite clumping / agglutination was considered as positive reaction, whereas no clumping/ agglutination was considered negative.

**Indirect-enzyme linked immunosorbant assay (i-ELISA)**

*Brucella* Antibody Test Kit, ELISA along with the user’s manual was procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) formerly Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS), Bangalore was used in the present study. The test was performed as per the protocol outlined in the user’s manual.

**RESULTS AND DISCUSSION**

Brucellosis is an infectious bacterial disease caused by genus *Brucella* and affecting a number of animal species. It is a worldwide zoonotic disease that is recognised as a major cause of heavy economic losses to the livestock industry and poses serious human health hazard (Ocholi *et al.*, 2005). Overall seroprevalence of brucellosis was detected, 8.70 and 7.41% in goats and sheep by RBPT and i-ELISA, respectively. Seroprevalence of brucellosis was 5.67 and 4.39% in goats and 12.26 and 10.97% in sheep by RBPT and i-ELISA, respectively.

**Comparative efficacy of serological tests**

In the present study, i-ELISA was found to detect low seroprevalence as compared to RBPT in goats and sheep. In goat, 4.39% of seropositivity was detected by i-ELISA against 5.67% by RBPT. On the other hand, 10.97% of seropositivity by i-ELISA and 12.26% by RBPT were detected in sheep. Overall, in both the animals, comparison to 7.41% of seropositivity was detected by i-ELISA as compared to 8.70% by RBPT. Similar results were observed by Rahman *et al.*, (2011) for testing of goats and sheep samples, who found highest seroprevalence of brucellosis by RBPT followed by STAT and i-ELISA. Din *et al.* (2013) found RBPT (11.33%) to be more sensitive than SPAT (9.33%) and STAT (7.66%) for testing goat samples. In contrast, Kotadiya (2012) found higher seropositivity of 11.38% by RBPT than 9.44% by STAT but the seropositivity of 18.20% by i-ELISA was highest as compared to these two tests for testing sheep samples from Gujarat. Sonawane *et al.* (2011) also observed higher seroprevalence of 15.60% by i-ELISA as compared to 5.92% by RBPT in samples of sheep and goat from Rajasthan.

Christopher *et al.* (2010); Godfroid *et al.* (2010) and Diaz *et al.* (2011) were of the view that these variations may be due to the ability of each test to detect different antibody classes.

**Comparison of sensitivity and specificity of i-ELISA and RBPT**

The comparative efficacy of RBPT to i-ELISA was determined with regards to their sensitivity, specificity and overall agreement in the diagnosis of caprine and ovine brucellosis for detecting antibodies, with regards to seroprevalence of *Brucella* infection.

In this study, the sensitivity of RBPT was 75.00% and specificity was 96.41% in goat (Table 1). Sharma *et al.* (2006) recorded slight lower sensitivity (67.85%) and higher specificity (99.51%) of RBPT in goat samples of Mehsana and Patan district of Gujarat when compared with dot-ELISA. Rahman *et al.* (2013) recorded the sensitivity (80.2%) and specificity (99.6%) of RBPT to be high in comparison to present study and Ekgatat *et al.*, (2010) also found higher diagnostic sensitivity (99.2%) and specificity (100%) of RBT. Reddy *et al.* (2014) found
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In the present study, overall the sensitivity and specificity of RBPT were found to be 80.00% and 97.01%, respectively as compared to i-ELISA (Table 3). Hence, i-ELISA was found to be a better serological test as compared to RBPT and could be advocated for screening of animals.

Table 1: Sensitivity, specificity and overall agreement of RBPT by comparing with i-ELISA for detection of Brucella antibodies in goat

<table>
<thead>
<tr>
<th>Test</th>
<th>i-ELISA</th>
<th>Total</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Overall Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBPT</td>
<td>18</td>
<td>13</td>
<td>31</td>
<td>75.00</td>
<td>96.41</td>
</tr>
<tr>
<td></td>
<td>06</td>
<td>510</td>
<td>516</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>523</td>
<td>547</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Sensitivity specificity and overall agreement of RBPT by comparing with i-ELISA for detection of Brucella antibodies in sheep

<table>
<thead>
<tr>
<th>Test</th>
<th>i-ELISA</th>
<th>Total</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Overall Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBPT</td>
<td>42</td>
<td>15</td>
<td>57</td>
<td>82.35</td>
<td>96.38</td>
</tr>
<tr>
<td></td>
<td>09</td>
<td>399</td>
<td>408</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>414</td>
<td>465</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Overall sensitivity specificity and overall agreement of RBPT by comparing with i-ELISA for detection of Brucella antibodies in goat and sheep

<table>
<thead>
<tr>
<th>Test</th>
<th>i-ELISA</th>
<th>Total</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Overall Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBPT</td>
<td>60</td>
<td>28</td>
<td>88</td>
<td>80.0</td>
<td>97.01</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>909</td>
<td>924</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>937</td>
<td>1012</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

low relative sensitivity (54.16%) while high specificity (100%) for RBPT. Hence, i-ELISA was found to be a better serological test as compared to RBPT and it could be advocated for screening of goat.

In case of sheep the sensitivity and specificity of RBPT were found to be 82.35% and 96.38%, respectively as compared to i-ELISA (Table 2).

Rahman et al. (2013) found similar sensitivity (82.8%) and specificity (98.3%) of RBT whereas Sharma et al. (2006) recorded lower sensitivity (55.55%) and similar specificity (94.59%) of RBPT for sheep samples from Mehsana and Patan districts of Gujarat as compared to dot- ELISA. Barbuddhe et al. (1994) found lower relative sensitivity and higher relative specificity of 42.85 and 100.00% of RBPT, respectively for goat samples when CFT was considered as gold standard test. Al-Mariri et al. (2011) found higher sensitivity (91%) of RBT for Syrian female sheep samples when CFT was considered as gold standard test. Kotadiya (2012) recorded lower sensitivity (65.83%) and higher specificity (100%) for RBPT, considering i-ELISA as a gold standard test for sheep samples. Hence, i-ELISA was found to be a better serological test as compared to RBPT and could be advocated for screening of animals.

In the present study, overall the sensitivity and specificity of RBPT were found to be 80.00% and 97.01%, respectively as compared to i-ELISA in sheep and goat (Table 3).

Hence, i-ELISA was found to be a better serological test as compared to RBPT for screening of animals. Almost similar results were obtained by Tayshete (2001) who found the sensitivity of RBPT to be 71.42% in contrast to this study in which specificity of RBPT was slight high (100%), considering i-ELISA as a gold standard test. On the other hand, Coelho et al., (2008) who found higher sensitivity (97.6%) and lower specificity (77.6%) values of RBT. Al-Gardia et al. (2011) noted higher sensitivity (89.04%) and specificity (99.06%) of commercial RBPT and Khalek et al. (2012) also recorded higher sensitivity (92.90%) and specificity (83%) for RBT.
In this study, overall agreement of RBPT with i-ELISA was 95.75% for samples from small ruminants (Table 3); 96.53% for goats (Table 1) and 94.84% for sheep (Table 2) respectively. Hence, i-ELISA was found to be better serological test as compared to RBPT and it could be advocated for screening of goats and sheep for brucellosis. Almost similar results were recorded by Sadhu et al. (2015) in small ruminants with overall agreement between RBPT and i-ELISA of 92.50% and concluded i-ELISA to be a better serological test as compared to RBPT and STAT. Ekgatat et al. (2009) conclude i-ELISA to be a simple and rapid test that was highly sensitive and specific for antibody detection and could be a reliable alternative for presumptive serological diagnosis of Brucella sp. infection in cattle and goats. Nielsen et al., (2005) also concluded that i-ELISA performed better than the c-ELISA and the FPA in goats. Sharma et al. (2006) recorded slight higher 97.49% concordance of RBPT with dot-ELISA in goats but slight lower (86.95%) in sheep.

In diagnosis of caprine and ovine brucellosis, the efficacy of RBPT and STAT was considered doubtful (WHO, 2004). As per WHO (2006), it should be noted that although the ELISA is more sensitive than the RBPT, but sometimes, it does not detect infected animals which are RBPT positive.

CONCLUSION

The present study indicate the brucellosis prevalent in Gujarat. However in view of consideration of cost, feasibility and reliability as field diagnostic test, RBPT has been found to be much cheaper, easier and convenient to perform than ELISA. According to sensitivity and specificity ELISA is more sensitive than the RBPT. Hence, i-ELISA was found to be better serological test as compared to RBPT and it could be advocated for screening of goats and sheep for brucellosis.

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REFERENCES


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