Comparative Efficacy of Lateral Flow Assay, RBPT, MAT and i-ELISA for Diagnosis of Bovine Brucellosis

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ABSTRACT

The present study compared efficacy of Rose Bengal plate test (RBPT), Micro-Agglutination Test (MAT), indirect Enzyme Linked Immunosorbent Assay (i-ELISA) and Lateral Flow Assay (LFA) for detection of Brucella antibodies in bovine serum samples. Of the 40 samples tested, 15 (37.5%), 18 (45%), 21 (52.5%) and 10 (25%) samples were confirmed positive with RBPT, MAT, i-ELISA and LFA, respectively. Sero-positivity was found highest by i-ELISA, followed by MAT, RBPT and LFA. Relative sensitivity, relative specificity, positive and negative predictive value and likelihood ratio of RBPT, MAT, LFA were calculated considering i-ELISA as standard. The agreement between tests was calculated by kappa statistics and almost perfect agreement was observed between i-ELISA and MAT (k= 0.85), substantial agreement between i-ELISA and RBPT (k= 0.61) and moderate agreement between i-ELISA and LFA (k= 0.46). Overall sero-prevalence of brucellosis in bovines in this study was determined to be 25% following the principle of serial testing and 55% following the principle of parallel testing. It can be concluded from the present study that i-ELISA is a better serological test compared to RBPT, MAT and LFA and could be advocated for routine sero-diagnosis of bovine brucellosis. However, considering the simplicity, economy and rapidity, MAT can be used as routine screening test.

Keywords: Bovine brucellosis, i-ELISA, MAT, RBPT

Brucellosis in cattle and buffalo is caused by Brucella abortus, a highly virulent species belonging to Genus Brucella. It is the largest bacterial zoonosis in the world with more than 5 lakh new reports of human cases per year (Papas et al., 2006). Bovine brucellosis is endemic in around 86 countries and prevalent in 13% of world’s total bovine population (Anon, 2012). This disease is difficult to control due to the extensive management of animals and causes huge economic losses, adversely affects the reproductive and productive potential of the animals leading to restrictions in the international trade in animal and animal products. The diagnosis of brucellosis at laboratory level is based on a combination of cultural, serological and molecular methods. Although culture and isolation has been suggested as gold standard for diagnosis of brucellosis (OIE, 2012), it has many limitations like time consuming, reduced sensitivity in chronic infections and requirement of suitable bio-safety cabinets for handling culture materials. Therefore, for easy, prompt and accurate diagnosis, a number of serological tests like RBPT, STAT, CFT and ELISA are being used for routine screening of livestock for brucellosis with certain advantages and disadvantages. The present study focuses on the comparative evaluation of lateral flow assay kit (commercially available), RBPT, MAT and i-ELISA for detection of Brucella antibodies in bovine serum.

A total of 40 bovine serum samples were collected from cows having history of reproductive disorders viz., abortion, retention of placenta, endometritis etc from dairy farms in Jhansi, Uttar Pradesh. The samples were stored at -20°C until used.

Four different diagnostic techniques, i.e. LFA, RBPT, MAT and i-ELISA were compared for detection of Brucella antibodies.

Lateral flow assay was carried out using Antibody Rapid Detection Test Kit (M’s Genomix Biotech) as per the manufacturer’s protocol. The appearance of deep
purple colour band both in the control and test line region indicated positive result; and pink colour band in the control and no apparent colour in the test line region indicated negative result. Negative result were confirmed in 20 min.

The colour antigen (B. abortus strain 99) obtained from Biological Products Division, ICAR-IVRI, Izatnagar was used for the test. RBPT was performed as per the protocol designed by OIE (2012). The agglutination result were interpreted as + to +++.

Microagglutination test (MAT) is a modified form of the standard tube agglutination test. The B. abortus plain antigen procured from Biological Products Division, ICAR-IVRI, Izatnagar was used for the test. The test was performed as per the protocol described by Raies Ul Islam et al. (2013). All the serum samples were tested with a minimum of 8 serial dilutions (1:10 to 1:1280). Controls were run using known positive and known negative sera. Interpretation of the results was based on the formation of an agglutination reaction at the bottom of the well. The sample showing a titre of 1:40 or more was considered as positive.

Indirect enzyme linked immunosorbent assay (i-ELISA) was performed as per the protocol described by Reddy et al. (2014). A strong positive control, moderate positive and negative control was included in each microtitre plate. The optical density (OD) measured at 492 nm in an ELISA reader (MICROSCAN MS5605A9) of strong positive control was used to calculate the per cent positivity (PP) value for test samples using the following equation:

\[ PP = \frac{\text{absorbance of test sample} - \text{absorbance of strong positive control}}{\text{absorbance of strong positive control}} \times 100. \]

Serum sample having PP value > 73 in cattle was taken as positive. A cut-off value of 73 was determined based on the results of the analysis of the mean and standard deviation (SD) of the total negative population in the study. The mean of the test values from uninfected animals + 1 SD was used as the rationale in deciding the cut-off for the i-ELISA (Jacobson, 1998).

The results of different serological tests were compared with the results of i-ELISA as a gold standard. The relative sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio of a positive test result and negative test result of LFA, RBPT and MAT were calculated using MedCalc Software. The agreement between these diagnostic tests was calculated to find out Kappa Value (Thrusfield, 2005).

In present investigation, comparative efficacy of 4 serological tests for detection of bovine brucellosis was studied (Fig. 1). The relative sensitivity, specificity, positive predictive value, negative predictive value, positive and negative likelihood ratio of LFA, RBPT and MAT in comparison to i-ELISA as standard are presented in Table 1.

RBPT is the most common test for screening of livestock against brucellosis, having high sensitivity and low specificity. In this study, 37.5% samples were tested positive by RBPT. Akhtar et al. (2010) recorded 26% and 43% seropositivity by RBPT in cattle and buffalo, respectively. Similarly, Raies Ul Islam et al. (2013) revealed 45.5% sero-positivity by RBPT while Reddy et al. (2014a) reported 14.92% sero-positivity by RBPT. A lower percentage of 6% and 5.15% have been reported by Chothe and Saxena (2014) and Reddy et al. (2014b).

![Fig. 1: Graph showing the result of different serological tests for diagnosis of bovine brucellosis.](image)

### Table 1. Relative efficacy of different serological test keeping i ELISA as a standard test

<table>
<thead>
<tr>
<th>Test</th>
<th>RBPT</th>
<th>MAT</th>
<th>LFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re. Sensitivity</td>
<td>66.67%</td>
<td>85.71%</td>
<td>47.62%</td>
</tr>
<tr>
<td>Re. Specificity</td>
<td>94.74%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Positive PV</td>
<td>93.33%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Negative PV</td>
<td>72.00%</td>
<td>86.36%</td>
<td>63.33%</td>
</tr>
<tr>
<td>LR Positive</td>
<td>12.67</td>
<td>∞</td>
<td>∞</td>
</tr>
<tr>
<td>LR Negative</td>
<td>0.35</td>
<td>0.14</td>
<td>0.52</td>
</tr>
<tr>
<td>Kappa Value</td>
<td>0.605</td>
<td>0.85</td>
<td>0.463</td>
</tr>
</tbody>
</table>
Results in the present study were read within 4 min so as to avoid false positive reactions that may arise due to formation of fibrin clots. In the present study one sample positive in RBPT was negative in i-ELISA, which may be due to naturally occurring non specific agglutinins in some animals (Garin-Bastuji et al., 1999). Six samples that failed to yield a positive outcome in RBPT were positive in rest 3 serological tests.

Standard tube agglutination test (STAT), a common serological test for brucellosis is time consuming, requires more quantity of antigen and serum and is costly for sero-epidemiological studies where large number of samples are involved. Hence, MAT was developed as a screening test and successfully used in population surveys (Brown et al., 1981). In the present study, MAT was found to be more sensitive than RBPT and LFA and detected 45% samples to be positive for brucellosis. Similar result of 47.75% was reported by Raies Ul Islam et al. (2013). However, Sareyyupoglu et al. (2010) reported 1.5% samples to be positive by MAT using 524 samples. MAT detected 3 samples positive which were negative by RBPT and 8 sample positive which were negative by LFA in the present study. All sample detected positive by MAT were also positive by i-ELISA, hence the relative specificity and positive predictive value for MAT is 100% and the likelihood ratio of positive test result is infinity indicating a perfect test.

Lateral flow assay is a ready to use kit, which can be used at the farmer’s doorstep for diagnosis. But, the present study revealed a low relative sensitivity (47.62%) of LFA in comparison to i-ELISA, however, the relative specificity and positive predictive value are 100% with infinity likelihood ratio of a positive test result. LFA detected 11, 8, 5 samples negative which were positive by i-ELISA, MAT and RBPT respectively. Shome et al. (2014) reported 51.36% of buffalo serum samples as positive by LFA and found the relative sensitivity and specificity to be 87.06% and 92.65%, respectively, in comparison to c-ELISA.

i-ELISA has been considered as gold standard by many workers to compare the result of other tests for brucellosis (Nielsen et al., 1996) and it is also considered as reference test because it has been reported to be highly sensitive and specific for detection of IgG, IgM, IgA Brucella antibodies in blood serum and CSF (Araj, 1989). In the present study, i-ELISA was found to be more sensitive than RBPT, MAT and LFA and detected maximum number of samples as positive. Three (7.5%) serum samples, which were positive by i-ELISA, were negative by rest 3 serological tests. Most of the samples positive by LFA, MAT and RBPT were also positive by i-ELISA. Similar findings were reported by many workers (Chachra et al., 2009, Ghodasara et al., 2010). A higher sero-positivity of 40.18% (Barbuddhe et al., 2003) and 22.01% (Varasada, 2003) have been reported using ELISA as compared to RBPT (16.80%) and STAT (14.03%) in cattle and buffaloes of Central Gujarat. In a comparative study, Ghodasara et al. (2010) could found the sero-positivity to be highest by i-ELISA (25%), followed by MAT (14.45%) and RBPT (10.56%). ELISA could eliminate false positive results and hence it has been preferred over RBPT and STAT for assessing the situation of bovine brucellosis in cattle (Erdenebaatar et al., 2004, Chand and Sharma, 2004).

Relative sensitivity of LFA, RBPT and MAT was found to be 47.62%, 66.67% and 85.71%, respectively, considering i-ELISA as gold standard test where as Relative specificity was found to be 94.74%, 100% and 100%, respectively. There was moderate agreement between i-ELISA and LFA (k = 0.46 at P < 0.05) substantial agreement between i-ELISA and RBPT (k = 0.61 at P < 0.05) and perfect agreement and between i-ELISA and MAT (k = 0.85 at P < 0.05) (Table 1). This suggests that MAT is a better serological test than LFA and RBPT for diagnosis of brucellosis. These findings are in agreement with Raies Ul Islam et al. (2013) and Sareyyupoglu et al. (2010). Ten samples were positive by all the 4 serological tests under study and hence as per the principle of serial testing, the overall sero prevalence in the samples is 25%. Similarly, 22 samples were positive by any one of the four serological tests under study, thus the overall sero prevalence in the samples is 55% as per the principle of parallel testing.

On the basis of the present study, it is concluded that bovine brucellosis is prevalent in dairy farms in and around Jhansi region, especially in the cases showing reproductive disorders. The present study revealed that although i-ELISA is a better serological test with high sensitivity and specificity it lacks cost effectiveness. However, considering the simplicity, economy reliability and rapidity, MAT can be used as routine screening test. But this needs further study and confirmation involving large number of samples both from diseased as well as healthy livestock.

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References


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