RESEARCH ARTICLE

Lateral Flow Assay as a Field Test for Sero-Diagnosis of Brucellosis in Small Ruminants

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Abstract

Lateral Flow Assay (LFA) considered as a field diagnostic test for brucellosis in small ruminant. For the purpose, 268 whole blood samples (sheep-112 and goats-156) were collected from selected organized and scattered herds located in South Saurashtra region of Gujarat. Out of 112 sheep tested, 12 (10.71%), 8 (7.14%), 15 (13.39%), while from 156 goats tested, 24 (15.38%), 18 (11.54%), 25 (16.03%) were positive by Rose Bengal Plate Test (RBPT), Indirect Enzyme-Linked Immunosorbent Assay (iELISA) and LFA, respectively. The sensitivity of RBPT as compared to iELISA was 80.00% and 84.00%; and LFA as compared to iELISA was 53.33% and 64.00% in sera samples of sheep and goats, respectively. The specificity of RBPT and LFA was 100% in sheep for both these tests, whereas in goats sera samples it was 97.71% and 98.47% as compared to iELISA. The negative predictive value for RBPT was 97.00% and 96.97%; while for LFA it was 93.27% and 93.48% in sera samples of sheep and goats, respectively. However, positive predictive values (PPV) for RBPT were 100.00% and 87.85%; while for LFA 100.00% and 88.89% in sera samples of sheep and goats, respectively. McNemar chi-square test for independent data (with Yates' correction) revealed non-significant difference between RBPT *vs* iELISA as 2.68% and 0.64%, while LFA *vs* iELISA as 6.25% and 4.45% in sera samples of sheep and goats, respectively. The concordance of iELISA with RBPT was k=0.874 and k=0.831; while for LFA k=0.664 and k=0.705 in sheep and goats, respectively. The performance of LFA is comparable to RBPT considering iELISA as gold standard, except sensitivity of LFA.

Keywords: Brucellosis, Indirect ELISA, Lateral flow assay, RBPT, Small ruminants.

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Introduction

Brucellosis is an infectious disease caused by Gram negative facultative intracellular bacteria of the genus *Brucella* which are pathogenic to a wide variety of animals and human beings. The disease has a considerable impact on human and animal health and socio-economy as rural income relies largely on livestock breeding and dairy products in our country. Microbiological isolation and identification of the organisms is the gold standard test, but it is expensive, laborious and has a limited sensitivity, and laboratory workers are at a great risk of acquiring the infection (Lopez-Merino, 1991). Many serological tests and their modifications have been developed by various workers from time to time to detect antibodies against *Brucella* organism, *viz.,* Rose Bengal plate test (RBPT), complement fixation test, milk ring test and enzyme-linked immunosorbent assay (ELISA). RBPT is routinely used sero-diagnostic test for the diagnosis of brucellosis in our country and it is a quick, cheap, effective and OIE recognized test for the diagnosis of brucellosis. However, it has disadvantages of reporting false negative results due to prozone phenomenon.

Lateral Flow Assay (LFA) test was introduced for the first time in the Brucellosis Research Laboratory of Bacterial Research Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria in July 2009 (Bertu *et al.,* 2010). It is a simple, reliable, field based pen side diagnostic tool and does not require much of technical skill, refrigeration and specific equipment for the diagnosis of many infectious diseases including brucellosis (Shome *et al.,* 2015; Kavya *et al.,* 2017). LFA can be used for testing animals in remote areas where access to laboratory facilities is difficult or when

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testing animals from nomadic or other migratory farmers (Abdoel *et al.,* 2008). Therefore, the present study was carried out to evaluate sensitivity and specificity of LFA and RBPT in comparison to indirect ELISA (iELISA) as gold standard test for sero-diagnosis of brucellosis in small ruminants.

Materials and Methods

Sample Collection

A total of 268 whole blood samples comprising of 112 from sheep and 156 from goats covering at least 10% of animals under flock, were collected from local farms in districts of Southern Saurashtra region of Gujarat. Serum samples were separated and stored at –20°C until used. All the animals were above six months of age and none of them were vaccinated against brucellosis as per available history. These samples were subjected to RBPT, iELISA and LFA for the screening of brucellosis (Table 1).

Rose Bengal Plate Test

RBPT antigen (Batch no. 1/20-21) was procured from the Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh. The test was performed according to procedure described by the manufacturer. Serum agglutination was considered as positive.

Indirect ELISA

Indirect multi-species ELISA test kit (NovaTec VetLine Brucella, Germany, Cat. No. BRUVT0050) was used to screen sheep and goats for detecting anti-brucella antibodies in serum. Before assaying all samples were diluted 1:100 with sample diluent. 100 µl each of controls and diluted samples were dispensed into wells. The plate was incubated for 1 hour at 37°C. After incubation the plate washed thrice with about 300 µl of washing buffer. Then 100 µl of VetLine Brucella Protein A/G conjugate was added to all micro wells except substrate blank well and incubated for 30 min at room temperature. After washing thrice, 100 µl of tetramethylbenzidine (TMB) substrate solution dispensed into all wells and incubated for 15 min at room temperature in dark. Finally, the reaction was stopped by adding 100 µl of stop solution in all the wells and plates were read on Thermo Scientific Multiskan GO Microplate Spectrophotometer at 450 nm filter to obtained optical density of the samples. The S/P% (sensitivity percent) was calculated using the following formula:

Table 1 : Comparison of LFA and RBPT with iELISA for brucellosis in sheep and goat

RBPT vs iELISA (Total 112 samples) for brucellosis in sheep					
Test (RBPT)	*Positive by iELISA*	*n*	*Negative by iELISA*	*n*	*Total*
Positive	True Positive	a = 12	False Positive	c = 00	a+c = 12
Negative	False Negative	b = 03	True Negative	d = 97	b+d = 100
Total		a+b=15		c+d=97	Total=112
LFA vs iELISA (Total 112 samples) for brucellosis in sheep					
Test (LFA)	*Positive by iELISA*	*n*	*Negative by iELISA*	*n*	*Total*
Positive	True Positive	a = 8	False Positive	c = 00	a+c = 8
Negative	False Negative	b = 7	True Negative	d = 97	b+d = 104
Total		a+b=15		c+d=97	Total=112
RBPT vs iELISA (Total 156 samples) for brucellosis in goats					
Test (RBPT)	*Positive by iELISA*	*n*	*Negative by iELISA*	*n*	*Total*
Positive	True Positive	a = 22	False Positive	c = 02	a+c = 24
Negative	False Negative	b = 03	True Negative	d = 129	b+d = 132
Total		a+b=25		c+d=131	Total=156
LFA vs iELISA (Total 156 samples) for brucellosis in goats					
Test (LFA)	*Positive by iELISA*	*n*	*Negative by iELISA*	*n*	*Total*
Positive	True Positive	a = 16	False Positive	c = 02	a+c = 18
Negative	False Negative	b = 09	True Negative	d = 129	b+d = 138
Total		a+b=25		c+d=131	Total=156

RBPT: Rose Bengal plate test, iELISA: Indirect enzyme-linked immunosorbent assay,
LFA: Lateral flow assay.
a= Number of samples positive to both conventional and the standard tests;
b= Number of samples negative to conventional but positive to the standard test;
c= Number of samples positive to conventional but negative to the standard test;
d= Number of samples negative to both conventional and the standard tests;
n= Number of samples.

NovaTec Units (NTU) = (Sample absorbance value x 10) / Cut-off value.

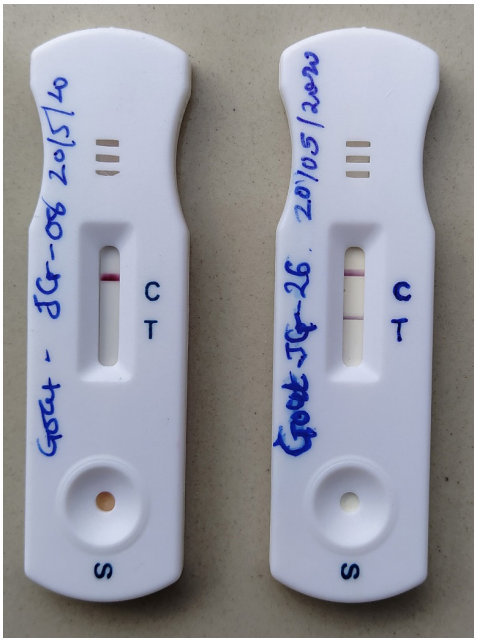
The cut-off is the mean absorbance value of the cut-off control determinations. Samples with NTU<9 were categorized as negative and NTU>11 were categorized as positive, where as an NTU: 9-11 were categorized as grey zone. The grey zone samples were subsequently retested by ELISA to classify either as negative or positive.

Lateral Flow Assay

A commercial quick VET Bovine *Brucella* antibody (Ab) lateral flow immunoassay kit (ubio Biotechnology Systems Pvt. Ltd., Cochin, Kerala, Cat. No. Q005-04) was used to screen animals for the presence of anti-brucella antibodies. Briefly, 10 µl of serum sample was added to sample well using a capillary tube and two drops of assay diluent were added over it. The test result interpreted at 10 min. In negative sample, the interpretation of the result was carried out as only control (single) line visible, while in positive sample two lines (control and test) were visible (Fig. 1).

Statistical analysis

The results of LFA and RBPT were compared with iELISA as gold standard because of its high specificity (Sp) and sensitivity (Se). Se and Sp of each test were calculated using MedCalc statistical software for Windows, version 19.3.1. Accuracies, Se and Sp of LFA and RBPT were statistically compared by McNemar's chi-square test using MedCalc software (MedCalc Software, Ostend, Belgium). The Sensitivity= a/(a+c), Specificity= d/(b+d), Positive predict value (PPV)= a/(a+b), Negative predict value (NPV)= d/(c+d) and Kappa statistic tests were calculated using MedCalc statistical software for Windows, version 19.3.1. (Table 1)



Negative **Positive**

Fig. 1: Lateral flow assay. C= Control line; T= Test line

Results and Discussion

The diagnostic test should be simple, rapid and sensitive for regular screening of animals for brucellosis. RBPT is widely used test for the diagnosis of brucellosis, but it often gives false positive results. ELISA has higher sensitivity and specificity, but laboratory equipment and technical skills are required to perform the test. Hence, in the present study, LFA was compared with RBPT and iELISA as the gold standard test.

The samples tested for RBPT, LFA and iELISA for sheep and goats were shown in Table 1. Out of 112 sheep tested, 12 (10.71%), 8 (7.14%), 15 (13.39%) were positive by RBPT, iELISA and LFA, while out of 156 goats tested, 24 (15.38%), 18 (11.54%), 25 (16.03%) were positive by RBPT, iELISA and LFA, respectively.

The sensitivity of RBPT *vs* iELISA was 80.00% and 84.00% and LFA *vs* iELISA 53.33% and 64.00% in sera samples of sheep and goats, respectively. The specificity of RBPT and LFA was 100% in sheep sera for both the tests, whereas it was 97.71% and 98.47% in goat sera as compared to iELISA (Table 2). RBPT test showed higher sensitivity as compared to LFA, however, specificity of both these tests was comparable. The present findings supported the Se and Sp of RBPT and LFA to iELISA reported in sheep and goats by earlier workers (Khalek *et al.,* 2012; El-Eragi *et al.,* 2014; Elshemey and Abd-Elrahman, 2014; Kotadiya *et al.,* 2015; Kavya *et al.,* 2016; Saadat *et al.,* 2017). Ahmed *et al.* (2016) reported a lower Se and Sp for both these tests. However, Trangadia and Prasad (2017) recorded lower Se and Sp of RBPT vs iELISA, but comparable Se and Sp of LFA vs iELISA in goats as compared to present findings. Higher Se and Sp of RBPT and LFA were reported by Rahman *et al.* (2013) and Hota *et al.* (2016). Conversely, Gusi *et al.* (2019) reported highest diagnostic Se of RBPT as compared to LFA and iELISA in sheep.

The negative predictive value (NPV) for RBPT was 97.00% and 96.97%, while 93.27% and 93.48% for LFA in sheep and goats, respectively. However, positive predictive values (PPV) for RBPT were 100.00% and 87.85%; while for LFA these were 100.00% and 88.89% in sera samples of sheep and goats, respectively. McNemar chi-square test revealed non-significant difference between RBPT vs iELISA as 2.68% and 0.64%, while LFA vs iELISA as 6.25% and 4.45% in sheep and goat sera, respectively. The concordance of iELISA with RBPT was k=0.874 and k=0.831; while for LFA it was k=0.664 and k=0.705 in sea samples of sheep and goats, respectively. The performance of LFA was comparable to RBPT considering iELISA as gold standard, except sensitivity of LFA (Table 2).

The LFA has shown good PPV and NPV greater than RBPT and almost similar to that of iELISA. Even though the sensitivity of LFA is lower than that of ELISA, it is almost closer to that of RBPT with specificity higher than RBPT. The higher specificity is optimal for minimizing the false positive results in the field conditions and proves that the LFA is a good test for sero-diagnosis in the field. As compared to the present

Table 2: Comparison of RBPT and LFA with iELISA for sensitivity, specificity and predictive values

	RBPT		*LFA*	
Statistic	*Sheep*	*Goats*	*Sheep*	*Goats*
Sensitivity (95% CI)	80.00% (51.91-95.67%)	84.00% (63.92-95.46%)	53.33% (26.59-78.73%)	64.00% (42.52-82.03%)
Specificity (95% CI)	100.00% (96.27-100%)	97.71% (93.45-99.53%)	100.00% (96.27-100%)	98.47% (94.59-99.81%)
Positive predictive value (95% CI)	100.00% -	87.85% (69.30-95.60%)	100.00% -	88.89% (66.22-97.03%)
Negative predictive value (95% CI)	97.00% (92.16-98.89%)	96.97% (92.87-98.74%)	93.27% (88.97-95.97%)	93.48% (89.47-96.03%)
Kappa statistics (95% CI)	0.874 (0.733-1.015)	0.831 (0.708-0.953)	0.664 (0.424-0.905)	0.705 (0.536-0.873)
Mc Nemar test Difference (95% CI)	2.68% (-1.05-2.68%)	0.64% (-2.80-3.55%)	6.25% (1.12-6.25%)	4.45% (0.38-8.59%)
Chi-square	1.3333	0.0000	5.1429	3.2727
Significance	P=0.2482	P=1.0000	P=0.0233	P=0.0704

RBPT: Rose Bengal Plate Test; iELISA: Indirect Enzyme-Linked Immunosorbent Assay
LFA: Lateral Flow Assay; CI: Confidence Interval.

findings. Shome *et al.* (2015) observed lower PPV and NPV values at organized buffalo farm. However, Hota *et al.* (2016) and Trangadia and Prasad (2017) reported comparable values for PPV and NPV in bovines and goats, respectively. Kappa values recorded in present study supported the findings of earlier workers (El-Eragi *et al.,* 2014; Elshemey and Abd-Elrahman, 2014; Kushwaha *et al.,* 2015; Kavya *et al.,* 2016). Ahmed *et al.* (2016) however reported comparatively lower kappa values than ours. Higher kappa values were also reported by some of the workers in past (Hota *et al.,* 2016; Kushwaha *et al.,* 2016) (Table 2).

Being costlier the LFA is probably not ideal for large-scale screening but could be a very useful tool to identify infected animals in smallholder herds, so that they can be removed or their milk is rejected or for providing public health advice to farmers following abortions in their herds. In general, serological methods used solitary carry the risk to interpret false negative results. Therefore, use of RBPT and the flow assay, or a combination of the two tests appears a good choice for countries such as India where brucellosis is endemic, but laboratory support is not readily available. This study also demonstrates the potential usefulness of this simple test to use in field based surveillance, which could be easily adopted without basic laboratory facilities.

Conclusion

Indirect-ELISA offers a significant advantage over conventional serological methods in the diagnosis of brucellosis in endemic region. Considering iELISA as a gold standard test, RBPT was more sensitive than LFA and the concordance of iELISA with LFA was comparable. The Lateral flow assay is a rapid point-of-care diagnostic test which makes it ideal for use in resource poor countries. LFA is an immuno-assay with high sensitivity and specificity which does not require expensive equipment, electricity and or refrigeration or special training. It could be used conveniently in the field and even on farms located in remote areas. In conclusion, LFA can be practically implemented in serological screening for brucellosis in small ruminants. However, evaluation on large sample size would be required for future use.

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