



Evaluation of immunochromatographic assay as a field test for the diagnosis of caprine brucellosis

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ABSTRACT

The present study was undertaken to assess the suitability of immunochromatographic assay (ICA) as a field test for the diagnosis of caprine brucellosis. Whole blood samples (570: 195 male goats, 375 female goats) were collected from selected organized herds and panjarapoles located in Southern Gujarat. Out of 570 animals tested, 19 (3.33%), 72 (12.63%) and 45 (7.89%) were positive by Rose Bengal plate test (RBPT), indirect ELISA (iELISA) and immunochromatographic assay (ICA) respectively. The sensitivity of RBPT and ICA vs iELISA was 26.39% and 61.11% respectively. Both the tests were 100% specific when compared with iELISA. Negative likelihood ratio for RBPT and ICA as compared to iELISA was 0.74 and 0.39 respectively. The negative predictive value for RBPT was 90.38% and 94.68% for ICA. However, the positive predictive values (PPV) for both the tests were 100%. McNemar chi-square test for independent data (with Yates' correction) revealed significant difference in the positive proportion between RBPT vs iELISA as 9.30% and ICA vs iELISA as 4.91%. The concordance of iELISA with RBPT was moderate ($k=0.385$) while it was good ($k=0.733$) for ICA.

Key words: Brucellosis, Goats, Immunochromatographic assay, Indirect ELISA, RBPT

The first outbreak of brucellosis in goats was reported in Mediterranean Island Malta by Zammit (1905). Ever since the discovery of the causative agent, brucellosis remains one of the most important and widespread zoonosis world over causing significant morbidity and enormous economic losses due to infertility, delayed oestrus, interrupted lactation and loss of off-springs, wool, meat and milk production. Clinical symptoms of brucellosis except abortion storm and histomorphological changes in a few organs are mostly non-specific. Thus specific diagnosis is usually based on a battery of laboratory tests. Microbiological isolation and identification of the organisms is the gold standard test. But it is expensive, cumbersome and has a limited sensitivity (Ray 1979). Further, laboratory workers are at a great risk of catching 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). Immunochromatographic assay (ICA) or lateral flow assay (LFA) is simple, reliable, field based pen side diagnostic tool and does not require much of technical skill, refrigeration

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and specific equipment for the diagnosis of many infectious diseases including brucellosis (Smits *et al.* 1999, Shome *et al.* 2015, Kavya *et al.* 2017). Therefore, the present study was carried out to compare ICA with RBPT and indirect ELISA as standard test.

MATERIALS AND METHODS

Sample collection: A total of 570 whole blood samples (195-male and 375-female) covering at least 10% of goats under flock were collected from selected organized herds and panjarapoles located in Southern Gujarat (Table 1). Serum samples were stored at -20°C until used. All these animals were above six months of age and as per the history available, none of these animals were vaccinated against brucellosis in past. The serum samples were subjected to RBPT, indirect ELISA (iELISA) and ICA for the diagnosis of brucellosis.

Rose Bengal plate test: RBPT antigen was procured from the Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh. The test was performed according to procedure described by the manufacturer. Briefly, 30 μl of serum was mixed with equal volume of *Brucella* antigen on white enamel plate circled approximately 2 cm in diameter with sterile glass or plastic rod. The result was recorded after the mixture was rocked gently for 4 min at room temperature. Any sign of agglutination was considered as positive.

Indirect ELISA: A commercial ID Screen® brucellosis

Table 1. Details of goats tested for brucellosis

Location	No. of goats tested		
	Male	Female	Total
Slaughter House, Surat Municipal Corporation, Surat, Gujarat	52	24	76
Al-Akbar Farm, Kanera, Navsari, Gujarat	72	05	77
Khadsupa Panjarapole, Khadsupa, Gujarat	23	69	92
Rata Panjarapole, Vapi, Gujarat	03	18	21
Nandini Panjarapole, Surat, Gujarat	00	36	36
An organized farm at Bharuch, Gujarat	27	141	168
Livestock Research Station, Navsari Agricultural University, Navsari	18	82	100
Total	195	375	570

serum indirect multi-species ELISA test kit (IDVet, France) was used to screen these animals for detecting anti-brucella antibodies. Briefly, 10 µl of test sera and controls were diluted by adding 190 µl of dilution buffer and incubated for 45 min at 21°C. Plates were washed thrice with about 300 µl of washing buffer. One hundred microliters of 1/10 diluted anti-ruminant IgG-peroxidase (HRP) conjugate was added to all the microwells and incubated for 30 min at 21°C. After washing thrice, 100 µl of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution was added to plates and incubated for 15 min at 21°C in dark. Finally, the reaction was stopped by adding 100 µl of stop solution in all the wells and plates were read at 450 nm filter to calculate optical density (OD) of the samples using Cyberlab ELISA-R01 Microplate reader (Cyberlab, USA). The S/P% was calculated using the following formula

$$S/P\% = \frac{OD \text{ sample} - \text{mean OD negative control}}{\text{Mean OD positive control} - \text{Mean OD negative control}} \times 100$$



Fig. 1. Immunochromatographic assay. C, control line; T, test line.

Samples with an S/P% ≤110 were classified as negative and ≥120 were classified as positive, otherwise classified as doubtful and were subsequently retested by ELISA to classify either as negative or positive.

Immunochromatographic assay: A commercial Anigen 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 at 20 min. In negative sample, only control line (single line) appeared while in positive samples, two lines (control and test lines) were seen (Fig. 1).

Statistical analysis: The results of ICA and RBPT were evaluated in comparison with iELISA (gold standard) due

Table 2. Comparison of ICA and RBPT with iELISA in 2×2 table

Test (RBPT)	RBPT vs iELISA (Total 570 samples)				Total
	Positive by iELISA	n	Negative by iELISA	n	
Positive	True Positive	a = 19	False Positive	c = 0	a+c = 19
Negative	False Negative	b = 53	True Negative	d = 498	b+d = 551
Total		a+b = 72		c+d = 498	

Test (ICA)	ICA vs iELISA (Total 570 samples)				Total
	Positive by iELISA	n	Negative by iELISA	n	
Positive	True Positive	a = 44	False Positive	c = 0	a+c = 44
Negative	False Negative	b = 28	True Negative	d = 498	b+d = 526
Total		a+b = 72		c+d = 498	

RBPT, Rose Bengal plate test; iELISA, Indirect enzyme-linked immunosorbent assay; ICA, Immunochromatographic 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.

to its high specificity (Sp) and sensitivity (Se) (Shome *et al.* 2015). Se and Sp of each test were calculated using MedCalc statistical software available online (http://www.medcalc.org/calc/diagnostic_test.php). Finally, the accuracies, Se and Sp of ICA and RBPT were statistically compared by McNemar's chi-square test using MedCalc software (Frank Shoonjans, V.7.2.1.0).

RESULTS AND DISCUSSION

The diagnostic test should be simple, rapid and reasonably 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 equipments and technical skills are required to perform the test. Hence, in the present study, ICA was evaluated considering iELISA as the standard test.

Out of 570 animals tested, 19 (3.33%), 72 (12.63%) and 45 (7.89%) were positive by RBPT, iELISA and ICA respectively. Comparison of ICA and RBPT with iELISA in 2×2 is given in Table 2. The Se of RBPT and ICA vs iELISA was 26.39% and 61.11% respectively. Both the tests were 100% specific when compared with iELISA. Negative likelihood ratio (NLR) for RBPT and ICA as compared to iELISA was 0.74 and 0.39, respectively with zero positive likelihood ratio (PLR) for both the tests. The negative predictive value (NPV) for RBPT was 90.38% and 94.68% for ICA. However, the positive predictive values (PPV) for both the tests was 100% relative to iELISA (Table 3).

McNemar chi-square test for independent data (with Yates' correction) revealed significant difference ($P < 0.0001$) in the positive proportion between RBPT vs ELISA as 9.30%, ICA vs iELISA as 4.91%. The concordance of RBPT with iELISA was moderate for RBPT ($k=0.385$) and of ICA with iELISA was good ($k=0.733$) (Table 3).

The present findings supported the Se and Sp of RBPT and ICA to iELISA reported in goats, sheep and cattle 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). However, a higher Se and Sp of RBPT and ICA were reported by Rahman *et al.* (2013) and Hota *et al.* (2016). On the other hand, Ahmed *et al.* (2016) reported a lower Se and Sp for both these tests.

Kappa values recorded in present study supported the findings by earlier workers (El-Eragi *et al.* 2014, Elshemey and Abd-Elrahman 2014, Kushwaha *et al.* 2015, Kavya *et al.* 2016) but could not support kappa values reported by Ahmed *et al.* (2016) which was comparatively lower than ours. Higher kappa values were also reported by some of the workers in past (Hota *et al.* 2016, Kushwaha *et al.* 2016).

Further, Shome *et al.* (2015) observed lower PPV, NPV, NLR and higher PLR values as compared to the present study during their study at organized buffalo farm. However, Hota *et al.* (2016) reported comparable values for PPV and NPV.

In conclusion, considering iELISA as standard test, ICA

Table 3. Evaluation of RBPT and ICA in comparison with iELISA

Diagnostic test	RBPT	ICA
Sensitivity (95% CI)	26.39% (16.70–38.10%)	61.11% (48.89–72.38%)
Specificity (95% CI)	100.00% (99.26–100.00%)	100.00% (99.26–100.00%)
Positive likelihood ratio (95% CI)	–	–
Negative likelihood ratio (95% CI)	0.74 (0.64–0.85)	0.39 (0.29–0.52)
Positive predictive value	100.00%	100.00%
Negative predictive value (95% CI)	90.38% (89.11–91.52%)	94.68% (93.01–95.96%)
Kappa statistics	0.385 (0.228–0.543)	0.733 (0.637–0.829)

RBPT, Rose Bengal plate test; iELISA, Indirect enzyme-linked immunosorbent assay; ICA, Immunochromatographic assay; CI, Confidence interval.

was more sensitive than RBPT and the concordance of iELISA with ICA was good but moderate with RBPT. ICA showed better negative predictive value than RBPT and lower negative likelihood ratio than RBPT. As ICA is simple, rapid and eliminates the requirements of training, it can be practically used for serological screening of goats against brucellosis. However, evaluation on large sample size would be required for future use.

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