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Comparative evaluation of blood based lateral flow assay for diagnosis of brucellosis in livestock species*

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ABSTRACT

Brucellosis is a zoonotic and economically important disease of livestock. Pen side diagnostics are the need of the hour for the rapid diagnosis of brucellosis. The present study aimed to evaluate blood based lateral flow assay (LFA) with serum based LFA and rose bengal plate test (RBPT) for diagnosis of brucellosis. Sera/blood samples [792: cattle, 153; buffalo, 55; sheep, 140; goats, 219; and pigs, 225] were collected from Karnataka, India with history of repeat breeding, retention of placenta and abortion.The seropositivity of brucellosis in cattle, buffalo, sheep, goats and pigs was 7.84, 3.63, 12.85, 2.73 and 39.11% by RBPT; 5.88, 3.63, 8.57, 2.73 and 31.1% by blood-LFA; and 7.18, 3.63, 10.71, 3.19 and 32.88% by serum-LFA. Relative specificity of blood based LFA was between 98.97 to 99.53% and 97.42 to 100% in comparison to serum-LFA and RBPT, respectively in all the species. The relative sensitivity for all the species ranged from 64.29 to 83.33% by blood-LFA and 57.14 to 84.09% by serum-LFA. The diagnostic accuracy of blood-LFA to RBPT and serum- LFA ranged from 91 to 99% among the species studied. It is opined that blood based LFA can serve as diagnostic test in place of serum based LFA for brucellosis screening in multiple livestock species and suggested for adaptation on-farm, slaughter, market, clinical and pre-purchase surveillance of brucellosis in the country.

Key words: Brucellosis, Blood-LFA, RBPT, Serum-LFA

Brucellosis remains one of the major public health concerns throughout the developing world accounting for an annual occurrence of more than 500,000 cases (Pappas *et al*. 2006). Based on epidemiological data, annual economic losses were estimated to be US$ 3.4 billion in India and these losses are in addition to the economic and social consequences of the disease in humans (Singh *et al.* 2015). The disease is manifested by reproductive failures, which include abortion, birth of unthrifty calves and retained placenta in female animals.

Although isolation of *Brucella* remains the gold-standard for the diagnosis of brucellosis, it is seldom carried out owing to lack of sensitivity and slow growth of *Brucellae*. The immunological tests like milk ring test (MRT), rose bengal plate test (RBPT), serum agglutination test (SAT) and enzyme linked immunsorbent assay (ELISA) are widely used to determine the disease status (Alton *et al*. 1988, Godfroid and Saegerman 2002).

Immunochromatographic lateral flow assays (LFA) were found useful as pen side tests for the diagnosis of many infectious diseases including brucellosis with appreciable sensitivity and specificity (Diaz *et al.* 1994, Smits *et al*. 1999, Irmak *et al*. 2004, Zeytinoglu *et al*. 2006, Abdoel *et al*. 2008, Sturenburg and Junker 2009, Genc *et al.* 2011, Elshemey and Abd-Elrahman 2014, Shome *et al*. 2015). The clinical materials such as serum, milk and blood samples were evaluated for various diseases including detection of anti *Brucella* antibodies.

Considering the possible advantages of testing blood, the present study aimed to evaluate LFA using blood sample instead of serum in comparison to conventional and widely used RBPT.

MATERIALS AND METHODS

Animals (792) were sampled from 15 different organised livestock farms with history of repeat breeding and abortion

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[cattle (153), buffalo (55), sheep (140), goats (219) and pigs (225)]. Approximately 5–10 ml of blood sample was collected from the jugular vein of bovines and small ruminants and 2 ml from ear vein of pigs using vacutainers with and without EDTA for serum and blood. The clotted blood tubes were centrifuged at 2,500 rpm for 10 min to obtain clear serum and stored at –20°C until tested.

Serum samples were subjected to RBPT using coloured antigen procured from the institute. Briefly, for the RBPT, undiluted serum sample (30 ml) was mixed with an equal volume of coloured antigen on a glass slide. The results were rated negative when agglutination was absent and 1+ to 3+ ratings as positive, according to the strength of the agglutination within 1 to 3 min. LFA for blood and serum cassettes provided by ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), using smooth lipopolysaccharide (sLPS) extracted from *B. abortus* S99 (procured from National Culture Repository, IVRI, Izatnagar, India) as per the OIE protocol (OIE 2012). Approximately, 10–15 µl of blood and serum samples were added to the sample port, followed by the addition of 2–3 drops of assay diluent and results were recorded within 3–5 min. Appearance of a coloured lane only in control slot was recorded as test negative and appearance of coloured lanes in both control and test slots was recorded as test positive.

The sensitivity and specificity of blood-LFA was compared with RBPT and serum-LFA using Med Calc statistical software (http://www.medcalc.org/calc/diagnostic_test.php).

RESULTS AND DISCUSSION

The diagnostic test should be quick, handy, cost-effective and reasonably sensitive for regular brucellosis screening in the herds. RBPT is widely used for its simplicity (Emmerzaal *et al.* 2002, Gall *et al.* 2004, Muma *et al*. 2007) but the test is often compounded with false positive results. Enzyme based assays (ELISA) have higher sensitivity and specificity but not suitable for field diagnosis as they require technical skill and equipment (Gwida *et al.* 2011). There is a great demand for simple and accurate tests for field use in the country. Hence, in the present study, standardized LFA was evaluated using blood sample and compared with serum sample in LFA to rule out the suitability of blood over serum sample for conducting LFA.

In the present study, alarmingly very high seroprevalence of brucellosis (39.11%) was recorded in pigs compared to other species. Introduction of an infected boar, poor management and biosecurity measures were perceived for the high disease prevalence. In India, seroprevalence upto 41% and 50% has been recorded in cattle and buffaloes, respectively with history of repeat breeding and abortion (Pandey *et al*. 2014). Overall prevalence of brucellosis in different species is depicted in Table 1. Of 792 animals, 127 (16.03%), 99 (12.55%) and 109 (13.76%) were positives by RBPT, blood-LFA and serum-LFA, respectively (Table 1).

Table 1. Seroprevalence of brucellosis in different livestock species

Species	No. of farms	No. of samples	RBPT (%)	Blood LFA (%)	Serum LFA (%)
Cattle	3	153	12 (7.84)	9 (5.88)	11 (7.18)
Buffalo	2	55	2 (3.63)	2 (3.63)	2 (3.63)
Sheep	4	140	18 (12.85)	12 (8.57)	15 (10.71)
Goat	3	219	06 (2.73)	06 (2.73)	07 (3.19)
Pig	3	225	88 (39.11)	70 (31.1)	74 (32.88)
Total	15	792	127 (16.03)	99 (12.55)	109 (13.76)

Blood-LFA detected 9 (5.88%), 2 (3.63%), 12 (8.57%) 6 (2.73%) and 70 (31.1%) whereas serum-LFA detected 11 (7.18%), 2 (3.63%), 15 (10.71%), 7 (3.19%) and 74 (32.88%) in cattle, buffalo, sheep, goats and pigs, respectively. In a study from Gujarat, India reported 13.33 and 9.32% prevalence in cattle and buffaloes, respectively using serum-LFA (Bhumika *et al*. 2015).

Blood-LFA revealed the relative specificity in comparison to RBPT between 98.97 to 99.53% and 97.42 to 100% to serum-LFA (Table 2). Similarly, relative sensitivity for all the species ranged from 64.29 to 83.33% in comparison to RBPT and 57.14 to 84.09% in comparison to serum- LFA (Table 2). The test showed good kappa agreement (0.7 to 0.82) in comparison to RBPT similar to that of serum-LFA for sheep, goats and pigs (0.75 to 0.86) whereas moderate kappa agreement was observed for bovines (0.56) (Table 2). The diagnostic accuracy ranged from 91 to 99% for both blood and serum-LFAs for all the species when compared to RBPT. The low sensitivity of 57.14 for blood-LFA could be due to interference by some of the blood constituents or due to fewer antibodies in blood compared to serum on volume basis. The sensitivity of blood-LFA was higher than that of RBPT in case of goat and swine. Whereas, specificity was found higher than RBPT in all the livestock species tested. High sensitivity and specificity of LFA had been reported (Smits *et al*. 1999,

Table 2. Comparative analysis of blood-LFA with RBPT and serum-LFA

Species	Bovines	Sheep	Goat	Swine
kappa value	0.70 (0.56)	0.70 (0.75)	0.82 (0.76)	0.82 (0.86)
Sensitivity (%)	64.29 (57.14)	61.11 (72.22)	83.33 (83.33)	78.41 (84.09)
Specificity (%)	98.97 (97.42)	99.18 (98.36)	99.53 (99.06)	99.27 (100.0)
PPV (%)	81.82 (61.54)	91.67 (86.67)	83.33 (71.43)	98.57 (100.0)
NPV (%)	97.46 (96.92)	94.53 (96.00)	99.53 (99.53)	87.74 (90.73)
Accuracy (%)	0.96	0.94	0.99	0.91

Figures in the parenthesis are the corresponding values for serum based LFA; PPV, positive predictive value; NPV, negative predictive value.

Irmak *et al*. 2004, Elshemey and Abd-Eirahman 2014, Abdoel *et al*. 2008, Khalek *et al*. 2012, Shome *et al*. 2015). The higher specificity is important as it minimizes the false positive results in the field conditions.

In conclusion, RBPT is widely used field test for brucellosis but test has issues of low specificity. Blood sample is mostly preferred than serum as separation and testing requires minimum laboratory facility. Hence, blood based LFA is perceived appropriate at the field level for the rapid detection of brucellosis. Further re-designing and fine tuning of in-house developed blood based LFA will facilitate wider applicability in the diagnosis of brucellosis.

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