*Haryana Vet.* (June, 2023) **62(1),** 50-53 ISSN 0033-4359 (P) **Research Article**

# COMPARATIVE EVALUATION OF FLUORESCENCE POLARIZATION ASSAY WITH VARIOUS SEROLOGICAL TESTS IN THE DIAGNOSIS OF BOVINE BRUCELLOSIS

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## ABSTRACT

Brucellosis is one of the highly contagious zoonotic diseases of livestock and human. The objective of this study was to assess the utilization and evaluation of Fluorescence Polarization Assay (FPA) technique for the serodiagnosis of bovine brucellosis in comparison to conventional serological tests. A total of 821 serum samples were collected from eleven districts of Tamil Nadu and subjected to RBPT, STAT and iELISA and FPA. Overall, the highest seroprevalence was encountered by iELISA (6.70%) followed by FPA (6.46%), STAT (4.38%) and RBPT (4.02%). In comparison with iELISA as a gold standard, diagnostic evaluation was performed for each serological assay. On diagnostic test evaluation, RBPT and STAT were identified as moderate agreement with iELISA whereas FPA had an almost perfect agreement with iELISA. In this context, FPA is found to be rapid, economical, more sensitive and specific for the detection of brucellosis and it can be better exploited as a diagnostic tool for laboratory and field level serological identification.

**Keywords:** Brucellosis, Diagnosis, Serology, FPA, Alternative test, bovine

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Brucellosis is one of the highly contagious bacterial zoonotic diseases and is characterized by abortion, retained placenta, repeat breeder, reduced milk yield and orchitis in animals as well as also in humans it produces multisystemic involvement affecting skeletal, neurological and reproductive issues (Kumar *et al.*, 2019; Singh *et al.*, 2019). The first brucellosis infection in cattle was reported on 1942 since then more number of epidemic reports were documented throughout the world. Though the disease is highly noted one due to its direct and indirect effect on animals and humans still the effective diagnostic idea is lacking. Since, each diagnostic test, has its own merits and demerits in the diagnosis of brucellosis, combination of test battle with high sensitivity and specificity is warranted to look over the national or wide level screening of animals (Naveenkumar *et al.*, 2018).

Fluorescence polarization immunoassay (FPA) is one of the promising tests and a homogeneous immunoassay useful for rapid and accurate detection of Brucella antibody in the sample. Due to its primary antigen-antibody interaction, the rate of reaction is very rapid and usually, a result may be obtained in minutes. FPA also can able to diagnose brucellosis from hemolyzed sera, milk samples and vaccinated animals (Rahman *et al.*, 2012). There are many numbers of researchers who standardized FPA technique in diagnosis of brucellosis against variety of species (Kalleshamurthy *et al.*, 2019). Owing to its importance in the diagnosis of brucellosis in the variety of species, bovine and swine species FPA is a new recommended OIE international trade test for the diagnosis of brucellosis (OIE, 2016). Because of these merits, FPA test is seeking its importance in the recent diagnosis of brucellosis. Based on the above facts the present manuscript aimed to utilize and apply FPA as a tool for serodiagnosis of bovine brucellosis from the unvaccinated and reproductive disorder population and to compare with traditional available serological diagnostic technique *viz*., RBPT, STAT and iELISA.

## MATERIALS AND METHODS

**Selection of animals:** Sexually mature cattle (n=821) were selected randomly from eleven districts of Tamil Nadu state in the present study with the history of abortion, retained fetal membrane, repeat breeding, infertility, pregnant and prepubertal anestrus heifers. Information on vaccination history was enquired from all the owners and only those animals that are not vaccinated against brucellosis were included in this study.

**Serum samples:** Blood samples (8-10 ml) were collected from 821 cattle by jugular vein puncture in sterile test tubes without anticoagulant and they were allowed to clot and then centrifuged at 3000 rpm for 15 minutes for serum separation. Separated serum was stored at -20°C until further use.

**Serological tests:** Rose Bengal test antigen and Standard tube agglutination test antigen were obtained from Indian

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Veterinary Research Institute (I.V.R.I), Izatnagar. The antigen was stored at 4º C until use. The RBPT and STAT were performed as per OIE, 2016.

**ELISA:** ELISA was performed using Svanovir Brucella-Ab indirect ELISA kit (SVANOVIR, Sweden) and the optical densities (ODs) were determined in a microplate spectrometer (Bio-rad) at 450 nm wavelength. Positive and negative control serum samples were included in each test. Interpretation of the results was based on Percent Positivity (PP) calculations; PP is calculated by (Test sample or negative control (OD) × 100) / (Positive control (OD)) and results were interpreted as positive for PP > 60 and Negative for PP < 60 for the individual serum (10 µl) sample.

**Fluorescence Polarization Assay (FPA):** Fluorescent polarization assay was performed at College Central Laboratory (CCL), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Haryana.

**Tracer preparation:** O-Polysaccharide from the smooth lipopolysaccharide of *B. abortus* was prepared as per standard procedure (OIE, 2016). The diluent used was 0.01 M Tris (1.21 g), containing 0.15 M sodium chloride (8.5 g), 0.05% Igepal CA630 (500 l) (formerly NP40), 10 mM EDTA (3.73 g) per litre of purified water, pH 7.2 ± 0.2 (Tris buffer).

**Protocol:** The FPA was carried out as per the procedure described in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016 (OIE, 2016) using Sentry® 200™ (ellie, USA). Briefly, 1 ml of diluent is added to a 10 × 75 mm borosilicate glass tube followed by 10 l of serum. It is important to mix well the contents of glass tube. A reading is obtained on the Sentry® 200™ to determine light scatter. A volume of antigen, which results in a total fluorescence intensity of 250-300 × 103, is added to the tube and mixed well. This volume may vary from batch to batch, but is generally in the range of about 10 l. A second reading is obtained on the Sentry® 200™ after incubation at room temperature (25°C ± 4°C) for approximately 2 minutes. A reading (in millipolarisation units, mP) over the established threshold level is indicative of a positive reaction. A typical threshold level is 90-100 mP units.

**Data analysis:** The efficacy of RBPT, STAT, FPA and iELISA in the diagnosis of brucellosis in dairy cows were assessed statistically as per the procedure of Snedecor and Cochran (1994). The sensitivity, specificity and Kappa value of various diagnostic tests were analyzed as per the methods described by Thrusfield (2018).

## RESULTS AND DISCUSSION

All the 821 sera samples were subjected to various serological test *viz.*, iELISA, FPA, STAT and RBPT which showed per cent positivity of 6.70 (55/821), 6.45 (53/821), 4.38 (36/821) and 4.02 (53/821) %, respectively. In this study the highest sensitivity in diagnosis of brucellosis was noted in both iELISA and FPA. Similar kinds of results with increased and equal sensitivity of iELISA and FPA in the diagnosis of brucellosis were reported by several researchers (Konstantinidis *et al.*, 2007 and Kalleshamurthy *et al.*, 2019). In this study, FPA revealed 6.45% of seroprevalence which is in agreement with Konrad *et al.* (2013) who have also documented a seroprevalence 6.4 % for brucellosis in buffalo population of Argentina. In contraray, Muma *et al.* (2009) who found higher positivity (20.66%) in Kafue Lechwe species whereas Weiner *et al.* (2010) (2.36%) and Rahman *et al.* (2012) (1.48% in buffaloes) found lower positivity than the present study. The attributable reasons for variation in prevalence might be due to sampling criteria variation, cutoff criteria and immunological status of animals included in the study. Among the field test for the diagnosis of brucellosis, RBPT is a suitable field test in determining the sero status of brucellosis throughout the world. However, RBPT test has some inherent lacuna to diffrentiate vaccinated from infected, cross-reaction with other bacterial pathogens and prozone formation. In this regard FPA is an effective field test in diagnosis of brucellosis which overcomes the above said limitations of RBPT (Poester *et al.*, 2010).

In comparison of routine serological tests *viz.*, RBPT, STAT and FPA with iELISA as gold standard were performed and reported the sensitivity (%), specificity (%) and kappa value respectively for RBPT (54.54, 99.60 and 0.66), STAT (61.81, 99.73 and 0.73) and FPA (94.54, 99.86 and 0.96) (Table 1). On comparison of RBPT with FPA and iELISA, our study also showed a higher sensitivity with FPA which again proves the utility of FPA as a routine field screening test for brucellosis.

Nielsen (2002) reported that RBPT and STAT, sensitivity varied from 21 to 98.3% and 29.1 to 100% and specificity varied from 68.8 to 100 % and 99.2 to 100%, respectively. In this research, RBPT and STAT sensitivity and specificity results were significantly corroborated with Nielsen (2002). However, Shome *et al.* (2006) reported a high sensitivity of RBPT> iELISA >STAT. These variations in results suggest us to use a minimum of two serological test battle in the diagnosis of brucellosis.

On comparison between FPA and iELISA, the sensitivity and specificity of FPA were 94.54 and 99.86 percent. Dajer *et al.* (1999) evaluated various diagnostic

**Table 1. Comparison and evaluation of STAT, RBPT and FPA with iELISA**

| Test | | iELISA | | Total | Sn (%) | Sp (%) | Kappa value test | Chi square |
|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | |
| RBPT | Positive | 30 | 3 | 33 | 54.54 | 99.60 | 0.6649 | (390.07)** P<0.01 |
| | Negative | 25 | 763 | 788 | | | | |
| Total | | 55 | 766 | 821 | | | | |
| STAT | Positive | 34 | 2 | 36 | 61.81 | 99.73 | 0.7331 | (463.78)** P<0.01 |
| | Negative | 21 | 764 | 785 | | | | |
| Total | | 55 | 766 | 821 | | | | |
| FPA | Positive | 52 | 1 | 53 | 94.54 | 99.86 | 0.9603 | (757.49)** P<0.01 |
| | Negative | 3 | 765 | 768 | | | | |
| Total | | 55 | 766 | 821 | | | | |

(*Sn- Sensitivity; Sp – Speciifcity; Chi square interpretation: ** - Highly significant, *- Significant, NS – Non significant 78; Kappa value interpretation: <0.00 – Poor agreement, 0.00 -0.20 – Slight agreement, 0.21 -0.40 –Fair agreement, 0.41 - 0.60 – Moderate agreement, 0.61-0.80 – Substantial, 0.81 - 1.00 – Almost perfect agreement)

tests for brucellosis and concluded that the specificity of FPA was 99.0% and sensitivity was 96.9%, whereas Gall and Nielsen, 2004 documented the same findings with FPA, test mean sensitivity was 97.5%, mean specificity was 98.9% and Performance Index was 196.4. Kappa value (0.9603) suggested that almost perfect agreement between these primary binding assays. This kappa agreement corroborated with Nicola *et al.* (2010) who documented 0.87 kappa values with FPA and iELISA. The comparative study conducted by Nielsen (2002) and opined that the sensitivity and specificity of FPA ranged from 99.0 to 99.3 and 96.9 to 100%, respectively in which sensitivity was slightly increased than present study. A comparative study was carried out by McGiven *et al.* (2003) between FPA and iELISA showed, 90.7% of test agreement. The agreement between our studies and earlier works on FPA were perfectly matched and Kappa value also showed almost perfect agreement with iELISA, which proves FPA as an effective alternate test for detection of brucellosis under field and in farm conditions. In general, FPA is significantly different with iELISA especially in differentiating vaccinal antibody and our study due to unvaccinated animal sampling the diagnostic performance of iELISA and FPA are more or less equal in status. The difference in sensitivity of iELISA and FPA might be due to cross reactional bacterial influence and serum sample status. FPA is having the advantage to eliminate the cross-reacting bacterial antibody and hemolysed sample also will produce a significant result which may be the reason behind the minor variation in sensitivity and specificity between FPA and iELISA (Rahman *et al.*, 2012).

## CONCLUSION

Brucellosis is one of the major endemic zoonotic diseases of animals in India. The national surveillance programs aimed to combat the epidemic curb of brucellosis. Identification of a single positive indicator of brucellosis will prevent economic losses that may lead to herd losses in organized sector. Results of our study clearly indicates that, FPA is an ideal, rapid, sensitive and low cost test which can be considered as a definite alternative test to iELISA in the serodiagnosis of bovine brucellosis in farm as well as laboratory conditions in developing nations like India.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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