

*Original Research***Comparative Evaluation of Various Diagnostic Tests in Diagnosis of Bovine Brucellosis**Viswanathan Naveenkumar¹, Mangalanathan Vijaya Bharathi^{2*} and Kannan Porteen³¹Department of Veterinary Preventive Medicine, Madras Veterinary College, TANUVAS, Chennai, INDIA²Cattle and Buffalo Breeding Unit, Post Graduate Research Institute in Animal Sciences, Kattupakkam, Kancheepuram 603 203, Chennai, INDIA³Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Chennai 600 007, INDIA*Corresponding author: mvijayabharathi74@gmail.com

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Abstract

Brucellosis is a zoonotic, economically important and contagious bacterial disease of livestock characterized by abortions, infertility and reduced production performance and diagnosis of brucellosis is so important in achieving appropriate brucellosis control strategy. This study was aimed to compare and evaluate various brucellosis diagnostic tests like Rose Bengal Test (RBT), Standard Tube Agglutination Test (STAT), indirect ELISA, Milk Ring Test (MRT) and milk ELISA in Tamil Nadu. A total of 483 sera and 483 milk samples were collected from 483 lactating cattle from various districts of Tamil Nadu. Overall, the highest prevalence was encountered by i-ELISA (6.62 %) followed by m-ELISA (5.59%), MRT (4.34%), STAT (3.93%) and RBT (3.10%). The evaluations of various diagnostic tests were achieved using i-ELISA as gold standard and the highest correlation between i-ELISA and m-ELISA (almost perfect agreement, k value - 0.82) were documented. This present study concluded that, m-ELISA have similar promising results with i-ELISA in detecting individual animals which implies, in lactating animals milk-ELISA can be used as suitable alternative test of serum-ELISA.

Key words: Brucellosis, ELISA, Lactating Cows, Tamil Nadu

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Introduction

Brucellosis is one of the contagious bacterial diseases in farm animal's which produces heavy economic losses due to abortifacient nature, infertility and reduction in productivity. Brucellosis is also a potent zoonotic disease and prevalent all over the world (Bhat *et al.*, 2012; Islam *et al.*, 2018). Throughout the world for effective brucellosis control, vaccination and test and slaughter policies were practised. In vaccine

application, due to accidental exposure risk to human or veterinarians, vaccinal antibody interference to diagnostic assays and abortifacient property of the vaccine strains, practical utility and usage under field conditions is still questionable. In India like countries, test and slaughter is not practically feasible, instead diagnose and reduce the exposure chance to other susceptible animals may find a way to reduce the incidence rate. In brucellosis, diagnostic choice of test is totally depends on the epidemiological purpose of the study.

In general diagnosis of brucellosis, by detecting antigen as direct approach (culture and polymerase chain reaction) and detecting antibody as indirect approach, in serum (Rose Bengal Plate Agglutination Test (RBPT), Standard Tube Agglutination Test (STAT), Enzyme Linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT) and Fluorescent Polarization Assay (FPA)) and in milk (MRT and milk ELISA) (Poester *et al.*, 2010; OIE, 2016; Mariam *et al.*, 2017; Kumar *et al.*, 2018) were followed. Even though, various diagnostic methods are available, culture is an irreplaceable gold standard technique in brucellosis (Godfroid *et al.*, 2010). In epidemiological surveillance study, due to fastidious nature of intracellular *Brucella* and laboratory acquired zoonotic risk, serological tests are highly recommended than isolation of *Brucella* sp. Because of merits and demerits of available diagnostic test in brucellosis diagnosis over each other (OIE, 2016), minimum of two gold diagnostic assays are recommended to decide the accurate status of disease which may direct us to planning effective disease control strategy in future (Kumar *et al.*, 2018a). This manuscript mainly focused: (1) to assess the prevalence of brucellosis from lactating cattle in Tamil Nadu by various diagnostic tests (2) to compare and evaluate various brucellosis diagnostic tests like Rose Bengal Test (RBT), Standard Tube Agglutination Test (STAT), indirect ELISA, Milk Ring Test (MRT) and milk ELISA.

Materials and Methods

Sampling Pattern

The present study was conducted in eleven districts of Tamil Nadu, India and 483 cows are randomly selected with the exclusion criteria as pregnant cattle. Blood samples (3 ml) and milk samples (10 ml from each quarter) were collected in aseptic manner using sterile coagulant added test tube (5ml) and sterile screw capped plastic vials (50 ml) respectively and transported on ice to the laboratory. Sera were separated as standard procedure and stored at - 20°C until further use.

RBT, STAT and MRT Test

RBT, STAT and MRT test antigen were obtained from Indian Veterinary Research Institute (I.V.R.I), Izatnagar. The antigen was stored at 4°C until use. As per Alton *et al.*, 1988, RBT, STAT and MRT were performed using respective samples.

Indirect ELISA and Milk ELISA Test

The *Brucella* antibody ELISA test kit was purchased from SVANOVIR, Sweden and used for testing 483 serum and 483 milk samples according to manufacturer's guidelines. The samples were run on Svanovir *Brucella*-Ab indirect ELISA kit and the optical densities (ODs) were determined in a microplate spectrometer (Bio rad) at 450-nm wavelength. Positive and negative control samples were included in each test. Interpretation of the results was based on per cent positivity (PP) calculations; PP is calculated by [Test sample or negative control (OD) x 100] / (Positive control (OD)) and results were interpreted as positive for PP \geq 60 and negative for PP < 60 for the individual serum (10 μ l) sample. In milk, results were interpreted as positive for PP \geq 10 and negative for PP < 10 for individual and pooled milk samples.

Statistical Analysis

The prevalence and associated diagnostic performance were assessed statistically as per method described by Snedecor and Cochran, 1994. As per Smith (1994) and Thrusfield (1995), evaluation of various diagnostic tests was analysed.

Results and Discussion

Prevalence of Bovine Brucellosis by Various Diagnostic Tests in Lactating Animals

In this cross sectional study, the highest prevalence was detected by i-ELISA (6.62%), followed by m-ELISA (5.80%), MRT (4.35%), STAT (3.93%) and RBT (3.10%) (Table 1).

Table 1: Prevalence of bovine brucellosis by various diagnostic tests in lactating animals

Prevalence by Test	Seroprevalence			Milk Prevalence	
	RBT Positive	STAT Positive	i-ELISA Positive	MRT Positive	m-ELISA Positive
Total no. of samples screened	483	483	483	483	483
Total no. of positive	15	19	32	21	28
Prevalence of brucellosis (%)	3.1	3.93	6.62	4.35	5.8

These results were concordant with NIVEDI (2014), with the results of i-ELISA (10.06%), MRT (7.38%), RBT (7.04%) and m-ELISA (6.04%). This study also concurred with Chand *et al.*, 2005 who conducted a similar study in Hisar, India with sheep samples and reported that i-ELISA (14.91%) detected more samples than m-ELISA (14.63%), RBPT (13.06%) and STAT (12.07%). Contradictory to present study by Salman and Nasri, 2012 and Neelam Kushwaha *et al.*, 2015 who found that m-ELISA detected more number of samples than i-ELISA. Deviation of this study with earlier workers might be due to presence or absence of antibodies in the samples from selected animals in various clinical and physiological conditions, problem in IgG transport from blood to milk against brucellosis and sample nature influence over each diagnostic

test (colostrums, mastitis milk, clotted milk and blood) (Chand *et al.*, 2005) and individual variations in diagnostic sensitivity and specificity of each test.

Comparison of Antibody Detection Methods in Serum and Milk Samples of Lactating Animals

Generally, the correlation of antibody level in milk and serum can be achieved only in milking animals. The i-ELISA and m-ELISA detected higher number positive of samples (6.62 and 5.80 per cent respectively), whereas the RBT, STAT and MRT detected much lower level of positivity in 483 lactating animals. In this study the ELISA techniques were detected more number of positive animals than other serological tests. For evaluation and comparison of various tests, i-ELISA was used as gold standard test. The sensitivity of RBT, STAT, MRT and m-ELISA when compared with i-ELISA were 34.37, 50.00, 46.87 and 78.12 per cent respectively, while the specificity of RBT, STAT, MRT and m-ELISA when compared with i-ELISA were 99.11, 99.33, 98.66 and 99.33 per cent, respectively. In a similar fashion Patel (2007) found the sensitivity of RBT, MRT and m-ELISA were to be 40.00, 30.00 and 55.00 per cent respectively and the specificity was to be 100, 93.93 and 87.87 per cent respectively. Contradictory to present study, Salmon and Nasri, 2012 reported a 92.8 and 98.8 per cent sensitivity and specificity of m-ELISA in cattle and Chand *et al.*, 2005 reported a 96.11 and 100 per cent sensitivity and specificity of m-ELISA in sheep with i-ELISA results.

Table 2: Evaluation of various diagnostic tests (serum and milk) with i-ELISA in lactating animals

Test		i-ELISA			Sensitivity (%)	Specificity (%)	Kappa value	Chi Square test
		Positive	Negative	Total				
RBT	Positive	11	4	15	34.37	99.11	0.4445	(111.35)** P<0.01
	Negative	21	447	468				
	Total	32	451	483				
STAT	Positive	16	3	19	50	99.33	0.6081	(192.44)** P<0.01
	Negative	16	448	464				
	Total	32	451	483				
MRT	Positive	15	6	21	46.87	98.66	0.5419	(149.03)** P<0.01
	Negative	17	445	462				
	Total	32	451	483				
m-ELISA	Positive	25	3	28	78.12	99.33	0.8223	(328.29)** P<0.01
	Negative	7	448	455				
	Total	32	451	483				

Chi square interpretation: ** - Highly significant, Kappa value interpretation: 0.41 - .60 – Moderate agreement, 0.61-0.80 – Substantial, 0.81 - 1.00 – Almost perfect agreement

In Kappa value assessment, agreement of m-ELISA (0.8223) with i-ELISA documented that almost perfect agreement between the two tests. In lactating animals, m-ELISA and i-ELISA showed similar sensitivity pattern which recommends that m-ELISA can be used for diagnosis of brucellosis in milk samples. The STAT (0.6081) agreement with i-ELISA found that substantial between the two test in field diagnosis of

brucellosis whereas, MRT (0.5451) and RBT (0.4445) agreement with i-ELISA were moderate in milking animals.

Conclusion

The present manuscript concluded that, ELISA techniques (i-ELISA and m-ELISA) are found to be higher sensitive than other conventional tests (RBT, STAT and MRT). In milking animals, m-ELISA may replace i-ELISA due to its high agreement value and promising results. Since, milk is a suitable non-invasive sample; in lactating animals m-ELISA may be useful as effective diagnostic tool in epidemiological surveillance study.

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