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Evaluation of PPD based ELISA in the Diagnosis of Bovine Tuberculosis

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ABSTRACT

541 animals from three dairy farms () were firstly screened for bovine tuberculosis by tuberculin skin testing, out of which 71 (13.12%) animals were found tuberculin reactors. The serum samples of 71 tuberculin positive-104 tuberculin negative and 363 non tuberculin tested animals were then evaluated by Purified Protein Derivative (PPD)-ELISA. PPD-ELISA yielded 57.74%, 8.65% and 24.24% seropositivity in tuberculin positive, tuberculin negative and non-tuberculin tested animals with an overall seroprevalence of 25.65% among tested sera. The relative sensitivity and specificity of ELISA with tuberculin test was 57.74% and 91.34%, respectively. The use of PPD based ELISA may be suggested in conjugation with tuberculin test for whole herd screening and culling programme especially in anergic state or advanced stages of infection.

Keywords: Bovine tuberculosis, tuberculin skin testing, PPD ELISA

The bovine tuberculosis is a major animal and public health problem since antiquity. It has maintained endemic levels in a number of countries including India. Histopathological findings, cultural isolation and molecular methods are useful diagnostic tools for bovine tuberculosis. In addition, techniques *viz.* gamma interferon assay, ELISA, lymphocyte proliferation methods etc. are carried out for research purpose which have their own diagnostic advantages and disadvantages. However, delayed type hypersensitivity based tuberculin skin testing is universally recognised and routinely employed technique for preliminary screening of herds as in majority of cases disease remains subclinical. With tuberculin testing false negative responses may be observed in recent infection, advance stage of the disease, animals with poor immune responses etc. Therefore, other ancillary tests are required to overcome these limitations.

The antibody-based assays like Enzyme-linked Immunosorbent Assay (ELISA) may assist in the detection of infected animals that test negative on tuberculin testing in the initial or advanced phases of infection (Whelan *et al.* 2008) and the same has been evaluated earlier by many workers (Lilenbaum *et al.* 1999a; Silva, 2001). So, it may be used as a complement to the tuberculin test, especially for anergic tuberculous cattle (Sayin and Erganis, 2013). With this view, the present study was aimed to evaluate the PPD based ELISA for serodiagnosis of bovine tuberculosis.

MATERIALS AND METHODS

Screening of dairy herds by tuberculin skin test

The present study was conducted in 541 dairy cattles at three dairy farms i.e. organized dairy farm-Bareilly (407), dairy farm-Mukteshwar (99) and Gaushala-Bareilly (35). The tuberculin skin testing was performed using PPD

Table 1: Testing of sera samples by PPD-ELISA

Sl. No.	Source of serum	Samples tested	Positive (%)
1	*Tuberculin positive	71	41 (57.74)
2	*Tuberculin negative	104	09 (8.65)
3	#Non tuberculin tested animals	363	88 (24.24)
	Total	**538**	**138 (25.65)**

* From tuberculin screened 3farms (541), #randomly collected samples of different farms (363)

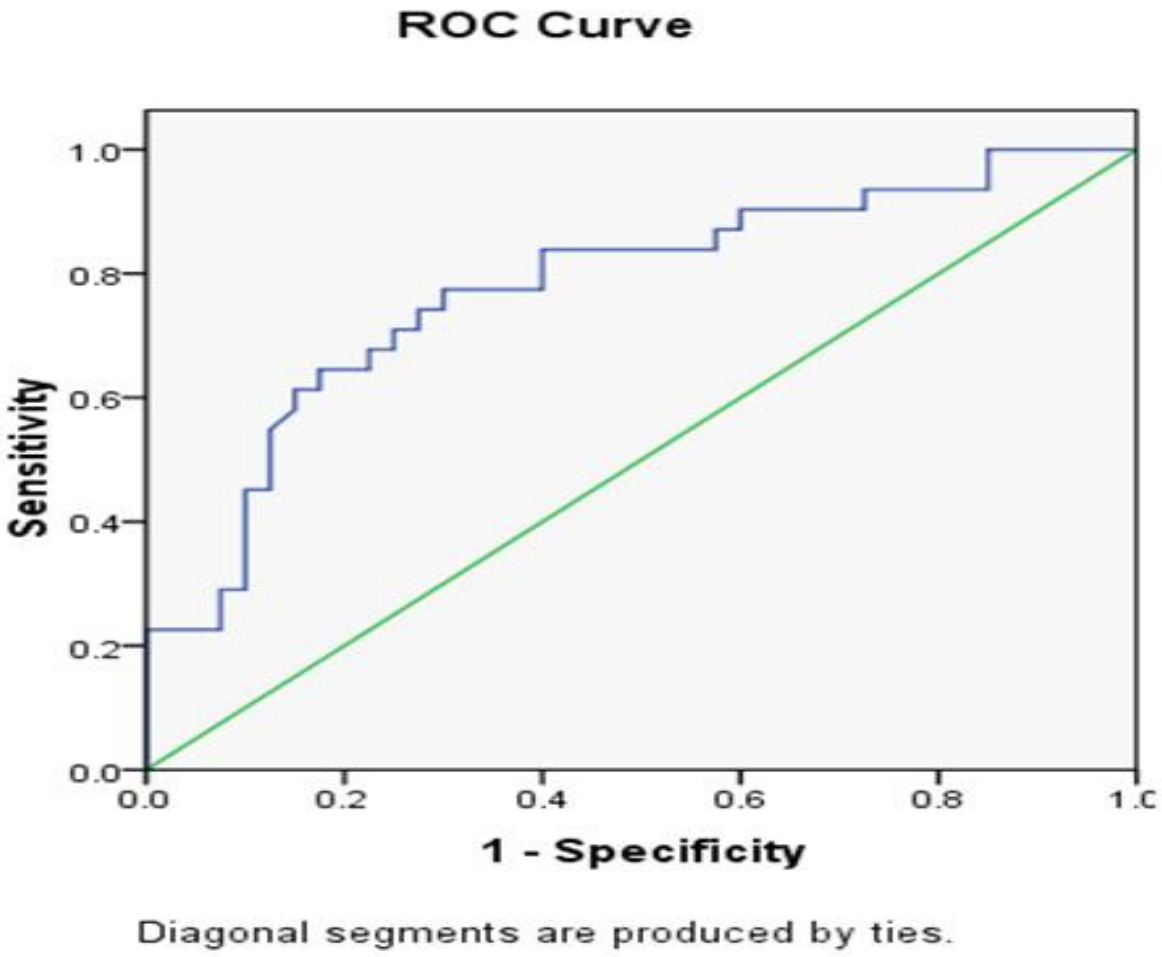

Fig. 1. Receiver Operator Curve (ROC) analysis

AREA UNDER THE CURVE

Test Result Variable(s): OD T

Area	Std. Error[a]	Asymptotic Sig.[b]	Symptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.776	.057	.000	.665	.887

Table 2: Source, sex, age and breed wise seroprevalence of bovine tuberculosis in various farms using PPD-ELISA

Sources of serum (No. of samples)	Male Positive/Total (%)	Female Positive/Total (%)	Total Positive/Total (%)	Age group 6mon-1yr Positive/Total (%)	1-3 yrs Positive/Total (%)	3-6 yrs Positive/Total (%)	>6yrs Positive/Total (%)
Organised dairy farms							
Farm-A, Bareilly (67)	—	37/67 (55.22)	37/67 (55.22)	—	0/1* (0.00)	3/11 (27.27)	34/55 (61.81)
Farm-B, Bareilly (164)	1/15 (6.67)	60/149 (40.26)	61/164 (37.19)	0/4 (0.00)	1/10 (10.00)	15/54 (27.77)	45/96 (46.87)
Mukhteshwar (73)	2/12 (16.67)	6/61 (9.83)	8/73 (10.95)	1/18 (5.55)	2/17 (11.75)	2/12 (16.66)	3/26 (11.53)
Rajnandgaon (64)	0/7 (0.00)	13/57 (22.80)	13/64 (20.31)	0/2 (0.00)	0/4 (0.00)	2/15 (13.33)	11/43 (25.58)
Lakhimpur (36)	3/36 (8.33)	—	3/36 (8.33)	—	—	0/7 (0.00)	3/29 (10.34)
Unorganised dairy farms							
Mathura (73)	—	8/73 (10.95)	8/73 (10.95)	—	0/1 (0.00)	2/12 (16.66)	6/60 (10.00)
Gaushala-Bareilly (35)	—	5/35 (14.28)	5/35 (14.28)	0/5 (0.00)	0/2 (0.00)	0/4 (0.00)	5/24 (20.83)
Ranchi (26)	—	3/26 (11.53)	3/26 (11.53)	—	0/2 (0.00)	0/5 (0.00)	3/19 (15.78)
Total (538)	**6/70 (8.57)**	**132/468 (28.2)**	**138/538 (25.65)**	**1/29 (3.44)**	**3/37 (8.10)**	**24/120 (20.0)**	**110/352 (31.25)**

Table 3: Comparative evaluation of PPD-ELISA and tuberculin skin testing

		Tuberculin skin testing		Total
		+	—	
PPD-ELISA	+	41	9	50
	—	30	95	125
Total		**71**	**104**	**175**

(Division of Biological Products, IVRI) by standard protocol (OIE, 2009).

PPD - ELISA

The serum samples of 71 tuberculin positive , 104 tuberculin negative and 363 non tuberculin tested animals were further evaluated by PPD-ELISA (Table 1 and 3). Blood samples were collected simultaneously during tuberculin testing followed by serum separation at the farm site and shipped thereafter on ice. The optimum concentration of bovine PPD (10 ng/well) was used as coating antigen and serum dilution was attained by the checkerboard titration method (Kumar *et al.* 1985). The ELISA was performed as described by Lilenbaum *et al.* (1999b) with some modifications.

Comparison of tests

The comparison between tuberculin skin testing and PPD-ELISA was done through 2×2 contingency table by calculating relative sensitivity, specificity, predictive value, true and apparent prevalence, accuracy, likelihood ratio of positive (LR^+) and negative tests (LR^-), diagnostic odd ratio (DOR), concordance and Kappa statistics (Thrusfield, 2005). The Receiver Operator Curve (ROC) analysis of serological response was also done to determine a cut off value for ELISA and to assess the test's performance (SPSS version 20).

RESULTS AND DISCUSSION

A total of 71 (13.12%) out of 541 animals were found positive from three dairy herds by tuberculin skin test. Herd prevalence based on tuberculin screening was higher in organized dairy farm-Bareilly (16.46%) followed by Gaushala (11.42%) while none of animals of dairy farm-Mukteshwar were found tuberculin reactor. Likewise more or less prevalence rate by tuberculin skin testing was reported by Thakur *et al.* (2010) and Ganesan (2012) in India. Higher prevalence in Dairy farm-Bareilly might be due to absence, or irregular screening and/or lack of segregation of tuberculin reactors. Absence of reactors at Mukteshwar farm may correspond to the geographical location of farm, managemental practices and concept of internal replacement of herd animals.

The overall seroprevalence of bovine tuberculosis by PPD-ELISA was 25.65% (Table 1) among tested sera samples. Seroprevalence rate was higher among tuberculin reactors (57.74%) followed by non-tuberculin tested animals (24.24%) and tuberculin negative (8.65%). There was a significant difference in seroprevalence among tuberculin positive, negative and non-tuberculin tested animals ($p<0.01$). The organised dairy farms showed overall higher seroprevalence as compared to unorganised farms (Table 3). In present study, PPD with concentration of 10ng/well was found sufficient to differentiate between positive and negative sera in ELISA which depicted high analytical sensitivity.

The epidemiological profile of bovine tuberculosis based on host characteristics (breed, sex, age etc.) and managemental factors was studied by analysing the potential risk factors associated with the disease. Among breeds seroprevalence rates in Brown swiss X Hariana crossbred, Tharparkar, HF cross, Jersey cross, Murrah, Jersey X Hariana crossbred were 40.00, 35.59, 30.86, 20.31, 19.69, 11.11%, respectively. None of the non-descript animals were detected positive. Seroprevalence rate differed significantly between HF cross and Jersey X Hariana crossbred; Jersey X Hariana crossbred and Brown swiss X Hariana crossbred; Jersey X Hariana crossbred and Tharparkar; Brown swiss X Hariana crossbred and Murrah ($p<0.01$); Brown swiss X Hariana crossbred and Jersey cross; Murrah and Tharparkar ($p<0.05$). Though exotic breeds are reported to be at higher risk compared to their native counterparts (Ameni *et al.* 2007) but higher seropositivity in indigenous cattle during present study might be due to poor husbandry practices on the farms. In comparison to cattle, the lower seroprevalence was found in buffalo (19.69 %), which is also corroborated with the findings of Gumber *et al.* (2003).

In sex wise analysis, female animals (28.20%) showed higher positivity as compared to males (8.57%). The higher seroprevalence in female animals might be correlated with large population size of female animals kept on farms as compared to male animals. More over males are also regularly screened for venereal diseases including tuberculosis which is mandatory. In addition,

a significance difference in seroprevalence was found between female animals of organised and unorganised farms ($p< 0.01$). Maximum sero reactivity (31.25%) was found in > 6 years age group animals in all farms. Sex wise and age wise similar trends were also reported by Trangadia *et al.* (2013). Possible reasons might be due to the chronic nature of disease and also protection by T cells which usually found predominantly in the circulation of young animals and the anti-mycobacterial immunity conferred by T cells of young animals (Stamp, 1948). The higher positivity in older groups can also be due to the slow progressive nature of disease.

Use of PPD antigen in ELISA during present work was to observe the response of PPD in cellular as well as humoral immune response. The antibody titre is generally inconsistent in initial stages of tuberculosis and rises in later stages of infection. As humoral response arises in later stages than the cellular response, the advanced and anergic cases can be detected by ELISA to rule out any leftover potential foci of infection in herds. The concept of direct application of ELISA was to pinpoint tentatively the most infectious foci present in farms so that further investigation can be directed by saving the time and resources. In addition, it is also beneficial when carrying out compulsory annual multiple diseases screening in large herds. On evaluation by 2 2 contingency table, the relative sensitivity, relative specificity, predictive value of positive test result, predictive value of negative test result, apparent prevalence, true prevalence, accuracy, LR^+, LR^-, DOR, concordance and kappa statistics of ELISA were 57.74%, 91.34 %, 0.82, 0.76, 28.57 %, 40.57 %, 77.71 %, 6.66, 0.462, 14.41, 0.777 and 0.515, respectively when compared with tuberculin skin testing (Table 3). Number of workers have presented different ranges of sensitivity and specificity of tuberculin skin testing with PPD-ELISA, which vary from 35-90% and 89-95% (Ritacco *et al.* 1987; Lilenbaum *et al.* 1999b; Silva, 2001), respectively. A relatively moderate concordance (0.777) was observed which indicated their usefulness as the tests for serial testing. In addition, in the absence of gold standard (culture isolation), the calculated kappa statistics also indicated the moderate agreement (0.515) between them. Furthermore, diagnostic odd ratio (14.41) also declared ELISA to be a moderately discriminatory in performance. Area under curve (Figure 1) and cut off value of ELISA in ROC analysis were 0.776 and 0.651, respectively. Present findings suggested ELISA as a moderately accurate test, since a perfect test yields an AUC of 1, whereas an uninformative test gives a value of 0.5 (Thrusfield, 2005).

ELISA has been observed to be moderately satisfactory test in association with tuberculin skin testing mainly in the second stage of herd screening for advanced and anergic cases which otherwise would have been negative in tuberculin screening. So, PPD-ELISA can be suggested as complement to tuberculin test for whole herd screening and culling programme especially in case when facility for culture isolation is lacking. However, a further more comprehensive study to evaluate and improve the efficacy of ELISA utilising different extracted and recombinant antigens for diagnostic purpose is suggested.

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