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Comparative Diagnostic Study of Brucellosis by RBPT and AB-ELISA

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Abstract

This paper deals with the seropositivity of brucellosis by RBPT and AB ELISA in cattle and buffalo as well as sensitivity and specificity of Rose Bengal Plate Test (RBPT) when compared with AB ELISA. The seropositivity was found to be 31.21 and 3.19 % by RBPT and 55.49 and 7.44 % by AB ELISA in cattle and buffalo, respectively. Sensitivity and specificity of RBPT was 49.49 and 93.24 % in cattle and 60 and 100 % in buffalo, respectively, when compared with AB ELISA as gold standard test.

Key words: Sero-diagnosis, Brucellosis, RBPT, AB ELISA, Cattle, Buffalo.

Introduction

Brucellosis is a widespread and economically important disease of animals. The disease is manifested by reproductive failure which includes abortion, retained placenta and subsequent infertility in female animals. Conventionally serological tests are used to screen or confirm the disease. RBPT is inexpensive, quick and highly sensitive, but not necessarily highly specific. The diagnostic value may be questionable for some tests on individual basis because of cross-reacting antibodies. Hence it is necessary to confirm by gold standard test like ELISA because correct and prompt diagnosis is important in controlling and eradicating the disease in animals.

Experimental

A total of 267 sera samples were collected at 21 days of abortion from 173 cattle and 94

buffaloes of Kaira district with history of abortion at six to eight months of gestation.

Collection of samples

The blood samples were taken in 9 ml clot activator vial (Greiner Bio-one, Austria) and kept in slanting position. The serum samples were collected in screw capped vials and stored at 4°C until used.

Rose Bengal Plate Agglutination Test

Brucella coloured antigen supplied by Division of Biological Product, IVRI, Izatnagar was kept at room temperature and shaken properly before use. Equal quantity of serum sample and RBPT antigen (40 l) were taken on a clean glass slide, mixed thoroughly with match stick and rotated clockwise and anti clockwise. The result was read immediately after 4 minutes. Definite agglutination was considered as positive reaction.

ELISA

Brucella Antibody ELISA Test Kit, along with the user manual procured from ADMAS, Bangalore and used in the present study. Immunochemical used are- Biotinylated antibovine IgG conjugates; avidine-HRPO conjugate

Reagents

- Hydrogen peroxide (H₂O₂)- Make 2% solution of H₂O₂ in double distilled water.
- Ortho-P Phenylene diamine dihydrochloride (OPU) chr- Omgen (sigma)- Dissolve one tablet of OPD (30mg) in 75 ml of double distilled water. Add 4 l of suspended H₂O₂ (2%) solution per ml of OPD.
- 1 M Sulphuric acid
- Carbonate bicarbonate buffer (pH 9.6 ± 0.2). Prepare following two solutions-
Solution A- Sodium carbonates (anhydrous) Dissolve 2.12 g in 100ml distilled water.
Solution B- Sodium bicarbonates- Dissolve 1.68 g in 100ml distilled water.
Working Solution (1× 0.05M) - Mix 7.0ml each of solutions A and B with 76.0 ml distilled water.
- Wash buffer- 0.002 M phosphate buffered saline (PBS) (pH 7.4 ± 0.2), Dilute 1 liter of PBS (0.01M) by adding 4 liters of distilled or de-ionized water plus 0.05% Tween 20.
- Blocking buffer- 0.01 M phosphate buffered saline, (pH 7.4 ± 0.2) plus 0.05 % Tween 20 plus 1% bovine gelatine.

Procedure

1:200 dilution of antigen was prepared in carbonate bicarbonate-buffer (pH 9.6 ± 0.2), 100 l of diluted antigen was added in each well of the ELISA plate (polysorp) and incubated at 40°C overnight. The plate was washed thrice with washing buffer and tapped against absorbent paper or towel. 100 l of 1:100 dilution of each test serum was added in blocking buffer and transferred to the test wells in the duplicate. Then 100 l of 1: 100 dilution of each control (C⁺⁺, C⁺ and C⁻) was added to respective control wells. Only blocking buffer was added in to C_c wells and incubated at 37°C for 1hr. Washing and drying procedure was repeated. 100 l of 1:250 dilution of biotinylated antibovine IgG

conjugate was added to blocking buffer in each well and then incubated at 37°C for 1hr. The washing and drying procedure was repeated again. Then 100 l of 1:250 dilution of Avidine Horseradish peroxidase conjugate was added to blocking buffer in each well and incubated at 37°C for 30 minutes. The washing and drying procedure was repeated again. 100 l of freshly prepared substrate chromogen solution containing OPD and H₂O₂ was added to all the wells in the test plate as well as in the first column of the block plate and incubated at 37°C for 15 minutes. 50 l of 1M H₂SO₄ was added to all the wells to stop the reaction and the plate was read at 492 nm in automated ELISA reader.

Interpretation

The median absorbance of the four strong positive control wells was calculated. Per cent Positivity (PP) of test serum and control was calculated as under.

$$PP = \frac{OD \text{ of test wells}}{\text{Median OD of } C^{++} \text{ well}} \times 100$$

PP value greater than 33 percent in test wells was considered as positive.

Results and Discussion

In the present study, the seropositivity was found to be 31.21 and 3.19% by RBPT and 55.49 and 7.44% by AB ELISA in cattle and buffalo, respectively (Table 1). Sensitivity and specificity of RBPT was 49.49 and 93.24% in cattle and 60 and 100% in buffalo, respectively, when compared with AB ELISA as gold standard test (Table 2). Previous study¹ also reported 33.33 and 56.02% seropositivity in bovine sera samples by RBPT and ELISA, respectively. They also reported sensitivity

Table 1. Seropositivity through RBPT and ELISA in cattle and buffalo

Species	RBPT positive	AB ELISA positive
Cattle	54 (31.21%)	96 (55.49%)
Buffalo	03 (3.19%)	07 (7.44%)

Table 2. Dichotomous table to work out sensitivity and specificity of RBPT by comparison with gold standard test

		ELISA Positive	ELISA Negative	Total	Sensitivity (%)	Specificity (%)
RBPT Cattle	Positive	49	05	54	49.49	93.24
	Negative	50	69	119		
	Total	99	74	173		
RBPT Buffalo	Positive	03	00	03	60	100
	Negative	02	89	91		
	Total	05	89	94		

and specificity of RBPT as 56.96 and 96.77%, respectively, when compared with ELISA. Our results closely corroborated previous observations^{2,3} who also reported that ELISA gave better results because chances of non-detection of infected animals in ELISA are minimum. This might have been due to ability of ELISA to detect all types of immunoglobulins⁴. Thus it is concluded that AB ELISA is more sensitive and specific than RBPT for detecting antibody of brucella organism.

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