Comparison of screening tests for detection of antibodies against brucellosis in different species of animals and human

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ABSTRACT : A total of 627 serum samples obtained from cattle (206), buffalo (79), sheep (09), goats (86), dogs (26) and humans (221) were subjected to a battery of brucellosis screening tests that included Rose Bengal plate agglutination test (RBPT), standard tube agglutination test (STAT), mercaptoethanol agglutination test (MET), heat-inactivation test (HIT), plate-enzyme linked immunosorbent assay (plate-ELISA) and dot-enzyme linked immunosorbent assay (dot-ELISA).The comparative field performance of these serological tests was examined. Results on overall performance of tests indicated that RBPT (15.08%) and plate-ELISA (10.36%) detected comparatively maximum number of reactors.

Key words: Brucellosis, diagnosis, serological tests

Brucellosis is one of the most important contagious diseases affecting animals and man across the globe. The disease is widespread in India (Li *et al*., 2017). In general, mass screening programmes in brucellosis have relied heavily upon the use of various serological tests in the target population especially in the absence of positive cultures (Radostits *et al*., 1994). Microbiological culture methods are technically challenging, time consuming leading to misdiagnosis, treatment delay, and severe morbidity and mortality (Mantur *et al.*, 2011). Tests based on serology measure the ability of the serum (antibody) to agglutinate with a standardised amount of killed *Brucella abortus* (antigen) containing O-side chain. These tests are safe but are prone to false-positive results due to other cross-reacting bacteria. Moreover, they are not useful in the detection of *Brucella canis* and *Brucella ovis* which lack the O-side chain (Kaltungo *et al*., 2013). Other useful tests include the direct smear examination, culture and isolation of the organism from blood or tissue samples, laboratory animal inoculation and more recent molecular techniques. Not all methods however are equally efficacious as measured in terms of sensitivity and specificity and can be used on its own to reliably detect the causative agent (Al Dahouk *et al.*, 2013). Therefore, diagnosis still relies on the combination of several tests to avoid false negative results (Poiester *et al*., 2010). Al Dahouk *et al*. (2003) strongly recommended at least two serological tests to be applied to avoid false results. The present study reports the results obtained by applying some brucellosis screening tests on serum samples collected from animals and human sources.

MATERIALS AND METHODS

Serum samples

Blood samples were collected from cattle, buffalo, sheep, goat, dog and humans. About 10-15 ml of blood (in humans, 3-5ml) was collected in a sterilized test tube to separate serum. The serum was stored at -20°C till use.

Antigens and Conjugates

The antigens (*Brucella abortus* plain antigen, abortus Bang ring antigen, Rose Bengal) were procured from the Division of Biological Products, Indian Veterinary Research Institute (IVRI), Izatnagar, U.P. *B.abortus* S99 sonicated antigen was provided by *Brucella* Lab, Division of Veterinary Public Health, IVRI, Izatnagar. The conjugates (goat anti-bovine HRP conjugate, protein A peroxidase conjugate, rabbit anti-goat HRP conjugate and goat anti-human HRP conjugate) for ELISA were obtained from the National Institute of Immunology(NII), New Delhi.

Serological tests

Rose Bengal plate agglutination test. The Rose Bengal plate agglutination test (RBPT) test was performed on a glass slide according to the method described by Morgan *et al*.(1969) using a drop of serum mixed with a drop of antigen. The results were read after 4 minutes.

Standard tube agglutination test. The standard tube agglutination test (STAT) was performed according to the procedure described by Alton *et al*. (1975). A titre of 80 IU/ml was considered positive for brucellosis. Positive test was characterized by the formation of flocculi in the test tube with clear antigen-serum mixture (supernatant).

Mercaptoethanol agglutination test. Mercaptoethanol agglutination test (MET) was performed as per the procedure described by Alton *et al.* (1975). A 0.1 mol/lit mercaptoethanol solution in normal saline was prepared. The dilution of serum was made in this solution instead of phenol saline.

Heat inactivation test. The heat inactivation test (HIT) was conducted according to the method described by Nagy and Sorheim (1969). The serum-antigen mixture was heated at 62°C for 15 minutes, cooled and centrifuged at 3000 rpm for 15 minutes. The supernatant was replaced by carbol saline. The test was considered positive if upon vigorous tilting of the tube, the serum-antigen mixture did not mix in the supernatant.

Enzyme linked immunosorbent assay. Enzyme linked immunosorbent assay (plate-ELISA) was performed on cattle, buffalo, goat and human serum samples according to the procedure described by Hudson and Hay (1991) and Chauhan (1995). The optical density (OD) of the test sample was measured at 492 nm wavelength on Microscan MS 5605 A ELISA reader. Samples exhibiting OD values of two or more times when compared to negative serum controls were considered positive.

Dot-enzyme linked immunosorbent assay. The dot-enzyme linked immunosorbent assay (dot-ELISA) was performed as per Batra *et al*. (1989) with little modification. The dipsticks were washed with 0.2 M phosphate buffer saline (pH 7.2) for 1-2 minutes and dried in air. A positive test was indicated by the appearance of a brown dot against a white background within one minute.

RESULTS AND DISCUSSION

A total of 627 serum samples from animals and human sources were screened. RBPT, STAT, MET, HIT, plate-ELISA and dot-ELISA were applied to examine the blood serum samples. In general, the RBPT yielded more positives when compared to other tests. In cattle, the test yielded 27.18 percent reactors followed by plate-ELISA (14.67%), STAT (10.32%), MET (9.28%) and HIT (6.52%). The prevalence of brucellosis as estimated by RBPT in goats, dogs and humans was also higher than that obtained through other tests. In buffaloes, however, RBPT (1.26%) and STAT (1.69%) detected nearly identical number of reactors. Wu *et al*, 2013 also revealed serological tests, STAT and RBPT to be useful methods for indirect diagnosis or screening of brucellosis and they found no significant difference between the two methods ($P>0.05$) in detection of brucellosis. Thus, RBPT was found to be a reliable rapid test for the diagnosis of brucellosis in animals. In fact, it (15.02%) was found superior to STAT (5.61%) as many animals tested negative by STAT were also detected positive by RBPT. The same observation of RBPT detecting more number of cases than STAT was also reported by Stemshorn *et al.* (1985) and Lorenzo and Pennimpede (1987). It had an added advantage in that it yielded result within 4-5 minutes and requires no elaborate laboratory facilities. The RBPT was also described by Heck *et al* (1980) as more sensitive than other more common sera antibody test. Plate-ELISA (14.67%) was not found superior to RBPT, the reason may be that the samples were held stored for a period of 4 months or more before conducting plate- ELISA. However, in case of specimens where the tests were run almost simultaneously, plate-ELISA proved to be a better test indicating that possibly storage of samples had affected the ELISA results. Observations regarding the factors affecting the efficiency of plate-ELISA had also been made by other investigators (Reynold, 1987; Nielsen *et al*., 1988 and Limet *et al*., 1988). In fact, Delgado *et al*. (1995) had indicated RBPT to be better than plate-ELISA. In our studies, plate - ELISA was found to be superior to RBPT in case of two species viz., buffaloes (6.7%) and goat (3.52%). Shafee *et al*, 2011 also indicated high sensitivity and specificity of ELISA compared to milk ring test. In our study, ELISA proved to be especially sensitive in humans (14.68%) where it detected much higher number of positives than STAT (1.14%) or RBPT (0.45%) making plate-ELISA a useful tool for detection of human brucellosis. Gomez *et al*., 2008 also found that sensitivity of ELISA was not better than conventional tests in cases of human brucellosis and Salman and El Nasri, 2012 concluded milk ELISA to be not very satisfactory as they yielded false positive reactors.

An overall view of all the tests performed indicated that RBPT was better in case of animals and plate-ELISA in humans (Table1). The performance of various tests when applied to total number of animal samples ,irrespective of the species, was as follows: RBPT (15.08%), plate-ELISA (10.36%), STAT (5.61%), MET (4.78%) and HIT (3.56%). Diaz *et al.* (2011) recommended RBPT to be the first very useful test for the diagnosis of human brucellosis being highly sensitive

Table 1: Field performance of various serodiagnostic tests against brucellosis

S.No.	Species	Percentage of total positive animals				
		RBPT	STAT	MET	HIT	Plate ELISA
1	Cattle	27.18 (206)	10.32 (184)	9.28 (183)	6.52 (184)	14.67 (184)
2	Buffalo	1.26 (79)	1.69 (59)	0.0 (59)	0.0 (59)	6.70 (59)
3	Sheep	0.0 (09)	0.0 (09)	0.0 (09)	0.0 (09)	ND*
4	Goat	2.32 (86)	0.0 (85)	0.0 (85)	0.0 (85)	3.52 (85)
5	Dog	7.69 (26)	0.0 (19)	0.0 (19)	ND	ND
6	Human	0.45 (221)	1.14 (175)	0.0 (163)	0.0 (163)	14.28 (175)

- ND-not done
- Figures in bracket denote number of serum samples screened

Table 2: Performance of four sero-screening tests on samples obtained from an organized herd

S.No.	Description	Dot ELISA	Plate ELISA	RBPT	STAT
1	Serum samples examined	70	70	70	70
2	Serum samples positive	31	23	34	15
3	Percent positive	44.28	32.85	48.57	21.42

and easily adaptable to test serum dilutions. They also concluded that depending upon the titer, a positive result does not need confirmation by other (usually more expensive, sophisticated and time consuming) tests. The test also revealed as a guide for screening animal brucellosis by Montasser *et al*., 2011. ELISA seemed to be a promising tool by Mantur *et al.* (2010) and Mantur *et al*. (2011).

The tests were also applied on 70 serum samples obtained from a herd with known history of brucellosis. RBPT and STAT detected 48.57% and 21.42% samples as positive, respectively. Dot-ELISA and plate-ELISA conducted on the same samples after 4 months of storage yielded 44.78% and 32.85% samples as positive (Table 2). RBPT and dot-ELISA appeared about comparable for the diagnosis of brucellosis. It was noticed that the samples with low titres (8-320 IU/ml) which were positive in RBPT were also positive in STAT. However, in samples with high titres (640 IU/ml or more), STAT, RBPT and plate-ELISA yielded comparable results as also noticed by Hussain *et al*. (1994).

CONCLUSION

Rose Bengal plate test (RBPT) was found to be a reliable screening test in most species of the animal screened. It was also able to detect reactors among animals considered negative by standard tube agglutination test (STAT) (i.e. titre values of 40 IU/ml).

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