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Comparative efficacy study of different serological tests for the diagnosis of brucellosis in bovines

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Abstract
The application of multiple serological assays currently available for the detection of *Brucella* antibodies in various species of animals indicates that no single test can detect all infected animals and therefore, combination of serological tests should include more sensitive tests designed to reduce the number of false negative reactions which contribute to the persistence of infection as a herd problem. So the presnt study was conducted to evaluate the comparative efficacy of RBPT and STAT to i-ELISA determined with regards to their sensitivity and specificity in the diagnosis of bovine brucellosis for detecting antibodies in cows and buffaloes. In present study seropositivity for *Brucella* antibodies were detected by i-ELISA, RBPT and STAT in 618 serum samples of cattle and buffaloes, comprising of 296 from cattle and 322 from buffaloes. Of these 12.46%, 20.06% and 35.92% of serum samples were found to be positive by RBPT, STAT and i-ELISA,, respectively showing higher efficacy i-ELISA followed STAT and RBPT.The sensitivity of RBPT and STAT was found to be of 34.68% and 55.86%, respectively, considering i-ELISA as a gold standard test while specificity was found to be of 98.48% and 98.99%, respectively. Thus, STAT was found to be more sensitive and specific than that of RBPT.

Keywords: Brucellosis, sensitivity, serological assays, Seropositivity

Introduction
Bovine brucellosis is found worldwide however it has been eradicated from many countries but it is one of the most serious diseases in developing countries. The rates of infection vary greatly from one country to another and between regions within a country. The highest prevalence is seen in dairy cattle. In India, brucellosis was first recognized in 1942 (Polding, 1942) [11] and is now endemic throughout the country. The disease has been reported in cattle, buffaloes, sheep, goats, pigs, dogs and humans (Chahota, *et al.*, 2003) [4].
Conventionally, serological tests are used to screen for or to confirm the disease. These screening tests are inexpensive, fast and highly sensitive but not necessarily highly specific. The most widely used serologicals tests for diagnosis of brucellosis in animals are Rose Bengal Plate Test (RBPT), Serum Tube Agglutination Test (STAT) and Enzyme Linked Immunosorbent Assay (ELISA). The diagnostic value may be questionable on individual basis because of likely presence of cross-reacting antibodies viz., *Salmonella, Yersinia spp.* and *E. coli* which leads to false positive diagnosis of brucellosis (Gallian *et al.*, 1998) [7], but for serological screening of herd with these tests remain ideal. So the presnt study was conducted to evaluate the comparative efficacy of RBPT and STAT to i-ELISA determined with regards to their sensitivity and specificity in the diagnosis of bovine brucellosis for detecting antibodies in cows and buffaloes.

Materials and Methods
Total 618 Bovines (cattle and buffaloes) sera samples were collected from the the villages of Banaskantha, Sabarkantha, Mehsana and Patan districts of North Gujarat for serodetection. About 9 ml of blood was collected aseptically from the jugular vein of individual animal in a vacuette with serum clot activator (Greiner bio-one, Austria). The vacuettes were kept in upright position at room temperature for about 2 hr. The separated serum was collected in a screw capped plastic vials and transported to the laboratory. The serum samples were heat inactivated at 56ºC for 30 min and merthiolate (1:10,000) was added in all vials as a preservative and sera were stored at -20ºC till further use. Collected serum samples were subjected to Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and ELISA.

Rose Bengal Plate Test (RBPT)
The antigen obtained from the Indian Veterinary Research Institute (I.V.R.I.), Izatnagar, Uttar Pradesh was used for Rose Bengal Plate Test. Definite clumping/agglutination was considered as positive reaction, where as no clumping/agglutination was considered as negative.

Standard Tube Agglutination Test (STAT)
The plain antigen obtained from the I.V.R.I., Izatnagar was used for the Standard Tube Agglutination Test (STAT).The degree of agglutination was judged by opacity of the supernatant and fluid highest serum dilution showing 50 per cent or more agglutination (50% clearing) was considered as the agglutination titre of the serum. 1:40 titre per ml or above was considered positive for brucellosis in cows and Buffalo (as per manufacturers instruction).

Indirect-Enzyme Linked Immunosorbant Assay for bovine serum samples.
Brucella Antibody Test Kit, ELISA along with the user manual was procured from Animal Disease Monitoring and Surveillance (ADMAS), Bangalore, was used in the present study. The test was performed as per the protocol outlined in the user manual.

Result and Discussion
Comparative efficacy of the serological tests
In present study seropositivity for *Brucella* antibodies were detected by i-ELISA, RBPT and STAT in 618 serum samples of cattle and buffaloes, comprising of 296 from cattle and 322 from buffaloes. Of these 12.46%, 20.06% and 35.92% of serum samples were found to be positive by RBPT, STAT and i-ELISA,, respectively showing higher efficacy i-ELISA followed STAT and RBPT (Table 1).
Similarly, Varasada (2003) [15] also found higher seropositivity by ELISA (22.01%) as compared to RBPT (16.80%) and STAT (14.03%) in cattle and buffaloes of Central Gujarat. Kanani (2007) [8] also recorded higher seropositivity by ELISA (8.25%) as compared to RBPT (5.67%) and STAT (7.22%) in breeding bulls of Gujarat. Patel (2007) [9] recorded higher seropositivity by ELISA (29%) as compare to RBPT (7.79%) and STAT (18.61%) in cattle and buffaloes of Gujarat. Similarly higher efficacy of i-ELISA was reported by Chakraborty *et al.* (2000) [5], Sarumathi *et al.* (2003) [13], Barbuddhe *et al.* (2004) [2], Chand and Sharma (2004) [6] and Bhattacharya *et al.* (2005) [3] in cattle and buffaloes.

Table 1: Serodetection by i-ELISA, STAT and RBPT in cows and buffaloes

Name of animals	Total	RBPT	STAT	i-ELISA
Cows	296	44 (14.86)	73 (24.66)	121 (40.08)
Buffalo	322	33 (10.23)	51 (15.84)	101(31.37)
Total	618	77 (12.46)	124 (20.06)	222 (35.92)

Figures in the parentheses indicate percentage

Comparison of sensitivity and specificity of i-ELISA, RBPT and STAT
The comparative efficacy of RBPT and STAT to i-ELISA determined with regards to their sensitivity and specificity in the diagnosis of bovine brucellosis for detecting antibodies in cows and buffaloes. Total of 618 sera were tested by i-ELISA and compared with RBPT and STAT. Cross tabulation of RBPT and STAT with i-ELISA, considering i-ELISA as a gold standard test were recorded as per Samad *et al.* (1994) [12] to determine relative sensitivity and specificity of RBPT and STAT, is presented in Table 2. The sensitivity of RBPT and STAT was found to be of 34.68% and 55.86%, respectively, considering i-ELISA as a gold standard test while specificity was found to be of 98.48% and 98.99%, respectively. Thus, STAT was found to be more sensitive and specific than that of RBPT.

Table 2: Sensitivity and specificity of RBPT and STAT by comparing with i-ELISA (gold standard test) for detection of *Brucella* antibodies

Test		i-ELISA		Total	Sensitivity (%)	Specificity (%)
		Positive	Negative			
RBPT	Positive	77	06	83	34.68	98.48
	Negative	145	390	535		
	Total	222	396	618		
STAT	Positive	124	04	128	55.86	98.99
	Negative	98	392	490		
	Total	222	396	618		

Similar results were obtained by Chakraborty *et al.* (2000) [5] who found higher sensitivity (88.61%) and specificity (98.59%) of STAT over RBPT with sensitivity (56.96%) and specificity (96.77%). Patel (2007) [9] revealed higher sensitivity of STAT (61.19%) over RBPT (25.35%), however, In contrast to present study Singh *et al.* (2004) [14] revealed sensitivity of RBPT (88.46%) much higher than STAT (46.15%), while specificity of STAT (98.31%) was found slightly higher than RBPT (97.75%) considering ELISA as gold standard. Similarly, Sarumathi *et al.* (2003) [13] also found higher specificity of STAT (90.59%) than RBPT (88.22%). Pati *et al*. (2000) [10] also concluded that ELISA was more sensitive than that of RBPT and STAT. Agrawal *et al.* (2007) [1] and Kanani (2007) [8] also observed ELISA as more sensitive than that of RBPT and STAT. Chand and Sharma (2004) [6] advocated the use of ELISA in comparison to RBPT and STAT for assessing the situation of brucellosis in cattle to have better results because chances of non-detection of an infected animal in ELISA are minimum.

Conclusion
Among the three serological tests employed for detection of *Brucella* antibody in serum of cattle and buffaloes, the highest positive results were obtained by ELISA (35.92%), followed by STAT (20.06%) and RBPT (12.46%). Hence, i-ELISA was found to be a better serological test and could be advocated for screening of animals. Although, there is further need of evaluation using serum samples from bacteriological isolation positive animal. In addition to this, various control as well as prevention programs should be started at the district, state and national levels for decreasing the incidence of brucellosis. For this an wide spread extension education campaign about risk factors of disease, economic and zoonotic importance of disease should be started among veterinary practitioners and

livestock owners particularly in the high-risk areas and regular sero-surveillance of the disease required to know the status of control and prevention programs.

References

1. Agrawal R, Mahesh kumar, Singh JL. Seroprevalence of brucellosis in Uttaranchal. Indian Vet. J. 2007; 84:204-205.
2. Barbuddhe SB, Chakurkar EB, Bale MA, Sundaram RNS, Bansode RB. Prevalence of brucellosis in organized dairy farms in Goa region. Indian J of Anim. Sci. 2004; 74:1030-1031.
3. Bhattacharya DK, Ahmed K, Rahman H. Studies on sero prevalence of bovine brucellosis by different tests. J Vet. Pub. Health. 2005; 3:131-133.
4. Chahota R, Sharma M, Katoch RC, Verma S, Singh MM, Kapoor V *et al*. Brucellosis outbreak in an organized dairy farm involving cows and in contact human beings in Himachal Pradesh, India. Veterinarski Arhiv. 2003; 73:95-102.
5. Chakraborty M, Patgiri GP, Sarma DK. Use of Rose Bengal Plate Test, Serum Agglutination Test and Indirect-ELISA for detecting brucellosis in bovines. Indian J Comp. Microbiol. Immunol. Infect. Dis. 2000; 21:24-25.
6. Chand P, Sharma AK. Situation of brucellosis in bovines at organized cattle farms belonging to three different states. Journal of Immunology and Immunopathology. 2004; 6:11-15.
7. Gallien P, Dorn C, Alban G, Staak C, Protz D. Detection of *Brucella* species in organs of naturally infected cattle by polymerase chain reaction. Vet. Rec. 1998; 142:512-514.
8. Kanani AN. Serological, Cultural and Molecular Detection of *Brucella* infection in Breeding Bulls. A Ph.D. thesis submitted to A. A. U., Anand, 2007.
9. Patel TJ. Serological, cultural and molecular detection of *Brucella* infection in bovines including quatification in milk by real-time PCR. An M.V.Sc. thesis submitted to A. A. U., Anand, 2007.
10. Pati US, Singh KP, Chsndra S, Kumar H. Detection of *Brucella* antibodies in buffalo sera. Indian J Comp. Microbiol. Immunol. Infect. Dis. 2000; 21:91-93.
11. Polding JB. Brucellosis in India. Indian J Vet. Sci. 1942; 13:27-34.
12. Samad A, Awaz KB, Sarkate LB. Diagnosis of bovine traumatic reticulo peritonitis I: Strength of clinical signs in predicting correct diagnosis. J Appl. Anim. Res. 1994; 6:13-18.
13. Sarumathi C, Reddy TV, Sreedevi B. Serological survey of bovine brucellosis in Andhra Pradesh. Indian Journal of Dairy Science. 2003; 56:408-410.
14. Singh G, Sharma DR, Dhand NK. Seroprevalence of bovine brucellosis in Punjab. Indian Vet. J. 2004; 81:620-623.
15. Varasada RN. Seroprevalence of brucellosis in cattle, buffalo and human being in central Gujarat. A M.V. Sc. thesis, submitted to Gujarat Agricultural University, Sardar Krushinagar, India, 2003.