Comparison of Rose Bengal Plate Agglutination, Standard tube agglutination and Indirect ELISA tests for detection of Brucella antibodies in Cows and Buffaloes

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Abstract

A total of 180 serum samples (107 cows, 73 buffaloes) from cases of abortion and various reproductive disorders were collected for detection of Brucella antibody by Rose Bengal Plate Agglutination Test (RBPT), Serum Tube Agglutination Test (STAT) and indirect- ELISA (i-ELISA). The overall prevalence of brucellosis by RBPT, STAT and i-ELISA were 11.21%, 16.00% and 24.30% in cows 9.59%, 12.33% and 26.03% in buffaloes respectively. Overall seroprevalence of Brucellosis in cases of abortion, R.O.P. by RBPT, STAT and i-ELISA were 11.32%, 16.04% and 32.08% respectively. When three serological tests were compared, seropositivity was found highest by i-ELISA (25%), followed by STAT (14.45%) and RBPT (10.56%). The results shows higher prevalence of brucellosis in cases of abortion and R.O.P., while at lower level from various reproductive disorders as detected serologically indicating endemicity of the infection in villages around Anand city, Gujarat.

Keywords: Brucella, Gynaecological disorders, Cows, Buffaloes.

Introduction

Brucellosis is an important disease of livestock species and wild animals widely prevalent in most of the developing countries. The disease causes a variety of reproductive disorders, viz., infertility, retained placenta, abortions, endometritis, etc. and resulting in heavy economic losses due to interrupted lactation and also due to loss of calves, wool, meat and milk production which are the main impediments to profitability. The disease has a significant health hazard in contact human beings.

The diagnosis of brucellosis can be based on cultural isolation, serological tests and in recent year by biotechnological techniques. Cultural isolation is time consuming, cumbersome and requires specialized laboratory personnel. Molecular based detection is rapid and sensitive require establishment of advanced laboratories and trained personnel. At present mainly serological methods are used for diagnosis of Brucellosis infection in India. The long-term serological studies at national level have indicated that 5% of cattle and 3% of buffaloes could be infected with brucellosis (Renukaradhya et al., 2002). After reviewing various serological tests no individual test found perfect, however, error could be minimized using the most reliable test, Nielsen (2002) and Gall and Nielsen (2004).

Looking to the present situation of high seroprevalence of brucellosis (8.8%) in India and (8.7%) in Gujarat (Renukaradhya et al., 2001), it is necessity to know the seroprevalence of brucellosis in cases of abortion, infertility, reproductive disorders and infections to prevent the spread of this disease. Thus aim of the present study was detection of Brucella antibodies from the cases of abortion and various gynaecological disorders using serological tests like RBPT, STAT and i-ELISA, and comparative efficacy of these serological tests.

Materials and Methods

A total of 180 serum samples were collected from 107 cows and 73 buffaloes having a history of various gynecological disorders like abortion, retention of placenta (R.O.P.), endometritis, metritis, infertility and repeat breeding for the presence of Brucella antibodies using serological tests viz. RBPT, STAT and i-ELISA. Among 180 serum samples, 106 serum samples were collected having history of abortion and R.O.P. and 74 serum samples collected having history of reproductive problems (metritis, endometritis, repeat breeders and infertility) from cows and buffaloes.

RBPT and STAT: The RBPT antigen and B. abortus plain antigen for STAT (IVRI, Izatnagar) were used. The RBPT and STAT were performed as described by Alton et al., (1988). Definite clumping/agglutination was

Table-1. Prevalence of Brucella antibodies in cases of abortion or reproductive disorders in cows and buffaloes

Animals	Abortion and R.O.P				Reproductive disorder(endometritis,metritis, infertility, Repeat breeding			
	Total	Positive (%)			Total	Positive (%)		
		i-ELISA	RBPT	STAT		i-ELISA	RBPT	STAT
Cow	68	19 (27.94)	8 (11.76)	11 (16.18)	39	7(17.94)	4 (10.26)	6 (15.38)
Buffalo	38	15(40.21)	4 (26.67)	6 (15.79)	35	4 (11.43)	3 (8.57)	3 (8.57)
Total % Positive	106	34 (32.07)	12 (11.32)	17 (16.04)	74	11 (14.86)	7 (9.46)	9 (12.16)

considered as positive reaction, where as no clumping/ agglutination was considered as negative for RBPT while serum titer of 80 IU or above were considered to be positive, 40 IU as doubtful and less than 40 IU as negative for STAT (Alton et al., 1988).

Avidin-biotin serum enzyme-linked immuno-sorbent assay technique: The enzyme-linked immuno-sorbent assay (ELISA) was performed with an avidin-biotin (AB) ELISA kit (PD-ADMAS, Bangalore) and the protocol provided by the developers was followed precisely.

Briefly, for antigen coating, the required volume of working dilution of smooth lipopolysaccharide (S-LPS) antigen was prepared in coating buffer (carbonate and bi-carbonate buffer), by adding 5 µl of S-LPS stock solution per ml of coating buffer. A total of 100 µl of working dilution of S-LPS was dispensed into all 96 wells of a micro-plate. After 1 hr of incubation at 37°C, the plate was washed three times with wash buffer, after which control and test serum samples (1:100 dilution of dilution buffer) were added. After 1 hr incubation at 37oC, the plate was washed three times with wash buffer. Two immunoconjugates, biotin-anti-bovine IgG and avidin-horseradish peroxidase working solution were added at a dilution rate of 1.25 µl and 2.5 µl, respectively, per ml of blocking buffer, the plate was washed 3 times after each steps. After washing 100ul of freshly prepared chromogen-substrate solution (5mg OPD tablet in 25 ml of distilled water and 80µl of 3% H2O2) was added and kept for colour development till 10-15 min. Finally the enzyme-substrate reaction was stopped by adding 5.5% H2SO4 and colour development was read at OD 492 nm using an ELISA plate reader (Multiskan plus, Sweden) and result were interpreted by calculating percent positivity (PP) are given below

Calculate the median absorbance of the four strong positive control wells. PP of test serum and controls are calculated as:

$$PP = \frac{[(OD \text{ of test wells} / \text{control wells})]}{\text{Median OD of C++ wells}} \times 100$$

The mean of any sample that gives a PP value of more than 33% is positive and below 33% is negative. On the other hand, when a sample shows a PP value of 33%, then it is sent for retest (As per manufacturers' instruction).

Results and Discussion

Objective of the study was to know the seroprevalence of Brucella antibodies from cases of abortion, R.O.P. and other various reproductive disorders of cows & buffaloes using above mention three different serological tests.

Serodetection of Brucella antibodies by RBPT and STAT: Out of 180 serum samples tested the prevalence of Brucella antibodies by RBPT and STAT were 11.21% (12) and 16.00% (17) in cows, 9.59% (7) and 12.33% (9) in buffaloes respectively by both the tests. The over all seroprevalence was found to be 10.55% (19) and 14.44% (26) by RBPT and STAT, respectively (Table 1).

In Gujarat, Varasada (2003) studied overall seroprevalence study of brucellosis in cattle and buffaloes of central Gujarat, and observed 16.80% and 14.03% of animals positive by RBPT and STAT, respectively. Patel (2007) obtained 7.79% and 18.61% seropositivity among cattle and buffaloes by RBPT and STAT, respectively. Similar Reports in other parts of country were also observed by Sharma and Saini (1995) who reported 8.69% and 14.61% prevalence in cattle and buffaloes respectively. In comparison to present finding higher prevalence of Brucella antibodies in other parts of the country were studied by

Table- 2. Showing comparison of i-ELISA, STAT and RBPT

Name of animals	Total	i-ELISA (%)	RBPT (%)	STAT (%)
Cows	107	26 (24.30)	12(11.21)	17(16.00)
Buffalo	73	19 (26.03)	7(9.59)	9(12.33)
Total	180	45 (25.00)	19 (10.56)	26 (14.45)

Table-3. 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(%)	OverallAgreement(%)	Positive	Negative
RBPT	Positive	19	02	21	42.22	98.52	84.44
Negative	26	133	159				
Total	45	135	180				
STAT	Positive	26	01	27	57.78	99.26	88.88
Negative	19	134	153				
Total	45	135	180				

Barbuddhe et al. (2004) revealed 37.38% and 36.45% positive for Brucella antibodies by RBPT and STAT, respectively in Goa. Genc et al. (2005) detected B. abortus antibodies at rate of 58.9% and 55.2% by RBPT and STAT, respectively.

Serodetection of Brucella antibodies by i-ELISA : In present study, seropositivity of Brucella antibodies by i-ELISA (s-LPS) were determined from total 180 serum samples tested from the cases of abortion and reproductive disorders, the overall seropositivity for Brucella antibodies by i-ELISA were 25% (45), of these 24.30% (26) in cows and 26.03% (19) in buffaloes.

In comparison to the present finding among the cattle and buffaloes slightly higher prevalence of Brucella antibodies observed by Chand and Sharma (2004), recorded 26.50% seroprevalence by ELISA, Similarly, higher seroprevelance was also observed by Patel (2007) using ELISA, revealed overall seropositivity of 29.00% with prevalence of 38.29% in cattle and 26.63% in buffaloes. Much higher seropositivity of 40.18% by ELISA has reported by Barbuddhe et al. (2004). In comparision to present study, lower values of seroprevalence of 22.01% with 24.12% in cattle and 19.12% in buffaloes, were reported by Varasada (2003).

Prevalence of Brucella antibodies in various cases of Gynaecological disorders: Prevalence of Brucella antibody in abortion, R.O.P. and reproductive disorder was studied using i-ELISA, RBPT and STAT. Among 180 serum samples, 106 serum samples were collected having history of abortion and R.O.P. and 74 serum samples collected having history of reproductive problems (metritis, endometritis, repeat breeders and infertility) from cows and buffaloes. Of these 106 serum samples, 34 (32.08%) samples by i-ELISA, 12 (11.32%) samples by RBPT and 17 (16.04%) samples by STAT were found positive Brucella antibodies while among the 74 serum samples, 11 (14.86%) serum samples by i-ELISA, 7 (9.46%) by RBPT and 9 (12.16%) by STAT were found to be positive for Brucella antibodies (Table 1).

Overall seroprevalence of Brucella antibodies from the cases of Abortion and R.O.P. (32.07%) by i-ELISA were found much higher then the cases of reproductive disorder (14.86%) by i-ELISA in cows and buffaloes (Table 1).

In comparison to the present study higher prevalence was reported by Sharma et al. (1990) who reported 62.26% positive reactor buffaloes having history of abortion at 6-9 months of pregnancy and suspected for brucellosis. Barbuddhe et al. (2004) found 40 (37.38%), 39 (36.45%) and 43 (40.18%) serum samples positive for antibodies against Brucella, by RBPT, STAT and AB-ELISA, respectively from the cases of abortion storms. Rao et al. (1999) noticed that dot-ELISA gave a high percentage of positive results (16.25% and 31.25%) followed by RPAT (11.5% and 16.25%) and STAT (8.75% and 15.00%) in graded Murrah buffaloes and cross bred cows, respectively. They concluded that dot-ELISA was a good screening test for detecting bovine brucellosis. In the present study, higher prevalence of Brucella antibodies was detected by i-ELISA test in comparison to RBPT and STAT, considering i-ELISA as a gold standard test.

Comparative efficacy of serological tests : In the present study, i-ELISA in conjunction with RBPT and STAT was employed to compare the efficacy, of these 25%, 10.56% and 14.45% of serum samples were found to be positive by i-ELISA, RBPT and STAT, respectively of cows and buffaloes (Table 2).

Similarly, Varasada (2003) 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. Similarly, higher seropositivity by ELISA as compared to RBPT and STAT were also recorded by Rao et al. (1999). Kanani (2007) 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) recorded higher seropositivity by ELISA (29%) as compared to RBPT (7.79%) and STAT (18.61%) in cattle and buffaloes. Similarly higher efficacy of i-ELISA was reported by Chakraborty et al. (2000), Sarumathi et al. (2003), Barbuddhe et al. (2004), Chand and Sharma (2004) and Bhattacharya et al. (2005) in cattle and buffaloes.

Comparison of sensitivity and specificity of i-ELISA, RBPT and STAT: The sensitivity of RBPT and STAT was found to be of 42.22% and 57.78%, respectively, considering i-ELISA as a gold standard test while

specificity was found to be of 98.52% and 99.26%, respectively. Thus, STAT was found to be more sensitive and specific than that of RBPT. Overall agreement of RBPT and STAT with i-ELISA was found to be 84.44% and 88.88%, respectively. Hence, i-ELISA was found to be a better serological test as compared to RBPT and STAT and it could be advocated for screening of animals (Table 3).

Similar results were obtained by Chakraborty et al. (2000) they found higher sensitivity (88.61%) and specificity (98.59%) of STAT over RBPT with sensitivity (56.96%) and specificity (96.77%). Patel (2007) revealed higher sensitivity of STAT (61.19%) over RBPT (25.35%), however, in contrast to the present study reported higher specificity of the RBPT (99.39%) than that of STAT (98.78%). In contrast to present study Singh et al. (2004) 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.

Paweska et al. (2002) suggested that ELISA could replace not only the currently used confirmatory CFT, but also other two routine screening tests, namely the RBPT and STAT. Gall and Nielsen (2004) after reviewing various serological tests, concluded that no individual test found to be perfect, however, error could be minimized using the most reliable test. Chand and Sharma (2004) 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. As per OIE (2004), i-ELISA should be considered more as a screening test rather than a confirmatory test for testing of vaccinated cattle or herds.

Since serological tests for diagnosis are more effective as herd tests than individual tests for ruminants, any samples that tests positive to screening test should be considered a positive reactor in absence of vaccination, but the samples collected at the time of abortion that test negative should not eliminate as negative for Brucella antibodies, paired serum samples after 21 days should be screen for confirmation of positive/negative reactor for brucellosis accompanies with isolation on selective medium.

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