Sero-Epidemiological and Therapeutic Aspects of Brucellosis (*Brucella Abortus)* in Cattle & Buffaloes

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ABSTRACT

The present study was envisaged to record the seroprevalence of brucellosis in cattle and buffaloes in Chhattisgarh, India by employing the three serological tests viz. Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and Indirect-Enzyme Linked Immunosorbent Assay (I-ELISA) and to compare their sensitivity and specificity. The study also aimed to assess the therapeutic efficacy of combination of long acting oxytetracycline and streptomycin in brucellosis infected cattle. A total of 250 serum samples; 176 from cattle and 74 from buffaloes were screened for presence of *Brucella* antibodies by RBPT, STAT and Indirect ELISA. The overall seroprevalence of brucellosis in Chhattisgarh state of India by RBPT, STAT and I-ELISA was 13.0% 19.8% and 31.2% respectively in cattle whereas 16.2%, 14.8% and 20.2% respectively in buffaloes. Cattle of >6 years age group showed highest seroprevalence followed by 4-6 years and lowest in 0-2 years age group. On the contrary, buffaloes of 4-6 years age group showed highest seroprevalence followed by >6 years age group. Seroprevalence was higher in crossbred than indigenous cattle and more in female animals in cattle and buffaloes. Sensitivity of RBPT and STAT was recorded 47.14% and 57.14%, while specificity was recorded 98.88% and 96.11% respectively. Thus, STAT was found to be more sensitive but less specific than RBPT. In this study, overall agreement of RBPT and STAT with ELISA was found to be 84.4% and 85.2% respectively. The therapeutic study of brucella infected animals revealed that long acting oxytetracycline and streptomycin combination had a significant decrease in the antibody titre on the 30th day of post treatment.

Keywords: Seroprevalence, Brucella, Cattle, Buffalo

Brucellosis results in huge economic losses in countries where it is prevalent, but the true magnitude of disease is not known. In India, brucellosis costs around Rs. 350 million in the form of food, animal and labour losses (Kunen, 1994). The brucellosis in cattle, usually caused by Brucella abortus and occasionally by Brucella melitensis and Brucella suis. It is characterized by late term abortion; infertility

and reduced milk production as a result of retained placenta and secondary endometritis and excretion of the organism in uterine discharges and milk. In bulls, acute or chronic infections of the reproductive tract may occur, causing orchitis, epididymitis, seminal vesiculitis, hygromas, particularly of the carpel joints, especially in chronically affected herds. Man who acquire infection through consumption of unpasteurized milk, undercooked or fresh meat and blood and handling of aborted materials and live fetuses without using protective gear, often manifest anorexia, headache, arthralgia and general malaise and less commonly have insomnia, sexual impotence and constipation.

For diagnosis of brucellosis various tests are employed with varying degree of sensitivity and specificity. The most reliable diagnosis of brucellosis is bacteriological isolation and identification of *Brucella* spp. (Alton *et al.* 1975, Cetinkaya *et al.*, 1999). However, bacteriological examination, though provides incontrovertible diagnosis of *Brucella* infection, isolation of *Brucella* organism is tedious, cumbersome, time consuming and also health hazardous to the laboratory workers thus it is generally not being followed as routine diagnostic procedure. Moreover, attempt to isolate *Brucella* from individual animals especially in chronic cases may not be always successful. Because of these reasons, serological tests are used for diagnosis of brucellosis. A number of serological tests have been developed to detect antibodies against *Brucella* organism for diagnosing the disease, *viz.*, Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Complement Fixation Test (CFT), Milk Ring Test (MRT), Enzyme Linked Immunosorbent Assay (ELISA) and Radio immuno assay (RIA) etc.

No systemic work has been carried out on prevalence of *Brucella abortus* infection in the dairy cattle and buffaloes in Chhattisgarh state of India. Therefore, in order to meet the government national policy, there is a need to measure the prevalence of *Brucella abortus* in the dairy cattle. This study will provide information about the status of the brucellosis in cattle and buffaloes in Chhattisgarh and will form the basis for future disease control policy for the disease in Chhattisgarh state of India. Considering the above facts, the present investigation was undertaken to estimate the prevalence and comparative study of different serological tests in cattle and buffaloes and therapeutic efficacy of combination of long-acting oxytetracycline and streptomycin for detection of brucellosis in cattle.

MATERIALS AND METHODS

Study areas

The study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh state of India during the period from April, 2010 to October, 2010.

Chhattisgarh is situated between 17-23.7° N latitude and 80.4- 83.38° E longitude in Central Eastern part of India. The general climate of Chhattisgarh state is dry-sub-humid type where the annual potential evapo-transpiration is slightly higher than the annual rainfall. The average annual rainfall of the region is around 1400 mm and about 90-95 % of this amount is received during south-west monsoon season (June-October). Winter condition set in from mid-November where the average minimum temperature starts falling below 15° C. The atmospheric humidity is very high (>79%) during monsoon months and starts decreasing from October onwards and reaches as low as 15-20% during peak summer months.

The total of 176 cattle and 74 buffaloes from organized and unorganized farms (individual livestock owner) in and around Durg district of Chhattisgarh.state were taken for the study.

Data collection and blood sampling

The data were collected after preparing a questionnaire to record the information related with species, breed, age, sex and farm management practices of the animals. Blood samples were collected from jugular vein using vacutainer (BD vacutainer™ UK) tubes (10 ml). All the blood samples were kept in inverted position in a box containing ice (4° C) and carried back to laboratory immediately. All the vacutainer tubes were kept in inverted position for 5-6 hours and then tubes were placed in normal position. Thus the clot sticked to the knob of the cap and serum was separated. This separated serum was centrifuged at 3000 rpm for 15 minutes to get clear serum, which was transferred to sterile screw capped vials. One (1) drop of sodium azide (1:10,000) was added in all vials containing serum samples. Then the sera were stored at -20°C till further analysis.

Serological tests

All the collected serum samples were subjected to Rose Bengal Plate Test (Morgan *et al.*, 1969) , Standard Tube Agglutination Test (Alton *et al.*, 1975) and Indirect ELISA (Hudson and Hay, 1991) for the present study. The degree of agglutination was judged by opacity of the supernatant fluid. The highest serum dilution showing 50 percent or more agglutination (50% clearing) was considered as the titre of the serum. The titre value in I.U. per ml was estimated to be double the reciprocal titre obtained in agglutination test by the following formula.

$$\textit{Titre value (IU/ml)} = \frac{\textit{reciprocal of titre} \times 1000}{500}$$

A titre of 80 I.U. per ml or above is considered positive for brucellosis in cows, while titre between 40 and 80 I.U. is considered doubtful. However, a titre of 20 I.U. per ml is taken as doubtful in breeding bulls (cattle as well as buffaloes) whereas 40 I.U. and above is considered positive for brucellosis in these animals.

The comparison of the sensitivity, specificity and overall agreement between the various tests were done by using the statistical formula given by Samad *et al.* (1994).

Therapeutic study

A total of eighteen cows were selected for the therapeutic study of long acting oxytetracycline and streptomycin. The animals were divided into three groups viz. A, B and C. Group A and group B each comprised of six seropositive cows selected randomly. Cows of Group A received both long acting oxytetracycline @ 20 mg/kg body weight intramuscular (i/m) at 72 hours interval for 5 times and streptomycin @ 25 mg/kg body weight i/m daily for 5 consecutive days. Group B cows received no treatment and served as infected control. Group C compromised of Six (6) seronegative cows for brucellosis for comparison and served as uninfected control group. The therapeutic efficacy of the treatment was assessed on the basis of decrease in antibody titre of treated animals through STAT. Antibody titres of all the animals were measured on day 0 (pre-treatment), 18 and 30 of observation period.

Statistical analysis

Comparative study of serological tests was done by the formula of Samad *et al*. (1994) using 2×2 contigency table. To know the effect of treatment, the data were analyzed by the t-test and analysis of variance with one way classification and Duncan's Multiple Range Test. (Snedecor and Cochran,1994).

RESULTS AND DISCUSSION

The present study was carried out to detect seroprevalence of brucellosis in bovines by detecting antibodies in serum by employing RBPT, STAT and Indirect ELISA, to compare efficacy of three serological tests and assess the therapeutic efficacy of combination of long-acting oxytetracycline and streptomycin in brucellosis infected cattle.

Seroprevalence of brucellosis

The seroprevalence of brucellosis in bovines was studied by RBPT, STAT and Indirect ELISA. Out of 250 samples 35, 46 and 70 samples were found to be positive for brucellosis by RBPT, STAT and I-ELISA, respectively. So, the overall prevalence of the disease in cattle and buffaloes was 14% by RBPT, 18.4% by STAT, and 28% by I-ELISA. Table 1.

Out of 176 cattle sera samples tested by RBPT, STAT and I-ELISA, 23 (13.0%), 35 (19.8%) and 55 (31.2%) samples respectively were found to be positive for brucellosis. While in buffaloes, out of 74 sera samples tested, 12 (16.2%), 11 (14.8%) and 15 (20.2%) by RBPT, STAT and I-ELISA respectively showed seropositivity for brucellosis. In the present study, seroprevalence of brucellosis was found to be higher in cattle, by STAT (19.8%) and I-ELISA (31.2%) than buffalo (STAT, 14.8%; I-ELISA, 20.2%). While the seroprevalence of brucellosis by RBPT in buffaloes (16.2%) was higher than that of cattle (13.0%) Table 1.

Table 1: Species and age wise seroprevalence of brucellosis in cattle and buffaloes.

Species	Age (yrs)	Tested	Positive (%)		
			RBPT	STAT	I-ELISA
Cattle	0-2	29	0 (0.0)	0 (0.0)	4 (13.7)
	2-4	57	4 (7.0)	9 (15.7)	16 (28.0)
	4-6	65	14 (21.5)	16 (24.6)	25 (38.4)
	> 6	25	5 (20.0)	10 (40.0)	10 (40.0)
Total		176	23 (13.0)	35 (19.8)	55 (31.2)
Buffalo	0-2	5	0 (0.0)	0 (0.0)	0 (0.0)
	2-4	27	2 (7.4)	1 (3.7)	2 (7.4)
	4-6	35	9 (25.7)	9 (25.7)	12 (34.2)
	> 6	7	1 (14.2)	1 (14.2)	1 (14.2)
	Total	74	12 (16.2)	11 (14.8)	15 (20.2)
	Overall	250	35 (14.0)	46 (18.4)	70 (28.0)

Figures in parenthesis () indicate percentage

Breed wise analysis of the data revealed that crossbred cattle had higher seroprevalence than indigenous cattle. In this study, indigenous buffaloes showed 16.2%, 14.8% and 20.2% seropositivity by RBPT, STAT and I-ELISA respectively. No crossbred buffaloes taken for the study. Table 1.

Species and age wise seroprevalence of brucellosis has been presented in Table 2. In this study, cattle > 6 years age group showed the highest seroprevalence followed by 4-6 years and 0-2 years age groups.

On the other hand buffaloes of 4-6 years age group showed the highest seroprevalence followed by > 6 years and 2-4 years age groups buffaloes shown in Table 2.

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

Test		ELISA		Total	Sensitivity	Specificity	Overall Agreement
		Positive	Negative				
RBPT	Positive	33	2	35			
	Negative	37	178	215	47.1	98.8	84.4
	Total	70	180	250			
STAT	Positive	40	7	47			
	Negative	30	173	203	57.1	96.1	85.2
	Total	70	180	250			

The overall seroprevalence of brucellosis in cattle was more in female animals; 19.1%, 28.3% and 40.8% as compared to that in males ; 0%, 1.7% and 10.7% by RBPT, STAT and I-ELISA respectively. Similarly, in buffaloes, females showed

higher seropositivity ;19.3%, 17.3% and 24.1% by RBPT, STAT and I-ELISA respectively with no seroprevalence in males.

Out of 10 farms studied, 5 farms were positive for seroprevalence of brucellosis. In none of the farm, vaccination against brucellosis was carried out. Managemental practices were good in all farms and the owners were well aware about the importance of the disease. Due to wide variation in the number of samples tested from different farms, comparison in this regard could not be made.

Comparative efficacy of diagnostic tests

In the present study, a total of 250 serum samples were tested for the presence of *Brucella* antibodies by RBPT, STAT and I-ELISA and comparative efficacy of these tests were also carried out. The details of the result in respect to sensitivity and specificity of RBPT and STAT, cross-tabulation of result with that of ELISA, considering ELISA as a gold standard are given in Table 3. In this study, in comparison to overall 28% seropositivity by ELISA, 14% and 18.4% of samples were found positive by RBPT and STAT respectively. Species wise also, ELISA showed the highest number of seropositive cases ; 31.2% in cattle and 20.2% in buffaloes than RBPT and STAT Table 2. This might have been due to the ability of ELISA to detect all types of immunoglobulins. STAT was found to be more sensitive ; 57.1% but less specific ; 96.1% than RBPT. In this study, overall agreement of RBPT and STAT with ELISA was reported to be 84.4% and 85.2% respectively. Therefore, ELISA was found to be a Gold Standard Test. Hence, it could be advocated as the most sensitive test for screening animals against brucellosis.

Therapeutic study

A total of 12 cows positive for brucellosis by all the three tests, RBPT, STAT and I-ELISA were randomly divided into 2 groups viz. A, B, each comprising of 6 cows. Cows of group A were treated with longacting oxytetracycline @ 20 mg/kg body weight at 72 hours interval for 5 occasions. Cows of group B received no treatment and served as infected control. Six (6) healthy cows grouped as C were also included in the study as uninfected (healthy) control. The mean of serotitres of antibodies are presented in Table-3.

Table 3: Effect of the therapeutic regimen on serum antibody titre (Mean ± SE) in *Brucella abortus* infected bovines.

Group	Observation period		
	0 day	18th Day	30th Day
A	1386.6 ± 256.8	1121.6 ± 464.7	130 ± 102.6[a]
B	1120 ± 327.9	1280 ± 286.2	1493.3 ± 356.9[b]
C	00.0	00.0	00.0

A= Cows infected and treated group; B = Cows infected group; C = Cows healthy group
Mean values with different superscript vary significantly (P d" 0.05)

Before treatment (on zero day), the serotitres of group A cows on STAT ranged from 640 to 2560 IU giving an average of 1386.6 ± 256.8 IU, whereas that of group B (untreated cows) was 320 to 2560 IU with an average of 1120 ± 327.9 IU. Treatment with longacting oxytetracycline and streptomycin combination resulted in significant decrease in the serotitres of group A animals when compared with zero day (pre-treatment) and the mean value was 130.0 ± 102.6 IU. However, the mean serotitre of group A animals on 18th day (1121.6 ± 464.7 IU) was comparable with 30th day value i.e. 130.0 ± 102.6 IU. During different observation periods, 3 out of 6 cows showed decrease in the serotitre (upto 7 fold dilutions) on eighteenth day of post treatment, whereas serotitre remain unchanged in 3 animals. On 30th day of post treatment, 1 animal became seronegative and rest 5 animals showed decrease in the serotitre which varied from 2 fold to 7 fold dilutions.

On the other hand, in group B (untreated) cows, 2 showed 1 fold increase and the rest remain unchanged on 18th day while on 30th day, 5 out of 6 animals showed escalating trend (increase in the serotitre from 1 to 2 fold) and the rest 1 remain unchanged. The mean serotitre on day 0 was 1120.0 ± 327.9 IU which increased gradually during different observation periods and became 1493.3 ± 356.9 IU on 30th day but the difference was not statistically significant between 0 day (1120.0 ± 327.9 IU) and 18th day (1280.0 ± 286.2 IU). When serotitres of treated (A) and untreated (B) groups were compared, it was observed that both were comparable on day 0 and day 18 (Table-3), but there was significant difference (P d" 0.05) in respect to serotitres of treated (A; 130.0 ± 102.6 IU) and untreated (B; 1493.3 ± 356.9 IU) groups on 30th day of observation. All the 6 cows of group C remained seronegative to brucellosis during the observation period.

In this study, brucellosis seroprevalence, comparative efficacy of the diagnostic tests and the associated therapeutic aspects were investigated in cattle and buffalo in and around Durg district of Chhattisgarh, India.

Our results regarding the seroprevalence of brucellosis in cattle and buffalo are in agreement with the reports of Sethi and Singh (1980) in Uttar Pradesh and Delhi (cattle; 11.29% and buffaloes; 7.20%), Sandhu *et al.* (2001) in Punjab (cows; 10.06% and buffaloes; 9.33%) who also reported slightly higher seroprevalence of brucellosis in cattle than that of buffaloes by ELISA. On the contrary, Sharma and Saini (1995) reported higher seroprevalence in buffaloes (14.61%) than in cattle (8.69%) in Punjab. In the present study, the prevalence of brucellosis among cows was higher than that reported by Sandhu *et. al* (2001) in Punjab (10.06%) but lower than that recorded by Bachh *et al.* (1988) in Kashmir (44%). Higher seroprevalence of brucellosis in cows than buffaloes might be attributed to extensive use of A.I. in cows.

Our findings with respect to breed variation corroborated the findings of Dwivedi and Kumar (2006) who reported that prevalence of crossbred cattle (12.50%) is higher than the indigenous (5.38%) in ruminants of sub Himalayan Kumaon region. Jain *et al*. (2006) also reported higher seroprevalence (12.50%) in crossbred cattle

than indigenous cattle (5.38%) in domesticated ruminants of Garhwal region of Uttaranchal state. This shows that the indigenous cows are comparatively resistant to bovine brucellosis and crossbred cows are less adapted to the hot and humid climate including management practices of the particular region. The intensive use of A.I. in crossbred animals may be a contributing factor for higher prevalence of brucellosis. As there was no crossbred buffalo, so comparative study in respect to breed in buffalo could not be made.

With respect to Species and age, the present results are in agreement with the observations of Thakur (2002) and Tamyo *et al.* (1997). On the contrary, Akakpo *et al.* (1984) observed higher seroprevalence of brucellosis in cows of 10 years or above age group. Higher prevalence of brucellosis in animals above 4 years might be due to the fact that this is the most suitable age for breeding. It might also be due to the fact that there is a marked decrease in immune status with the advancement of age.

Sex wise our findings were similar to that of Ahmed and Abd-El-Aal (1997) who also reported slightly higher incidence (2.61%) in females than in males (0.59%). The higher prevalence of brucellosis in females may be due to the preferential localization of *Brucella* organisms in uterus having erythritol which stimulates growth of these organisms (Bala and Siddhu, 1982). While Bandara and Mahipala (2002) reported lower seroprevalence in bovine males (3.6%) than females (4.9%), Kanani (2007) also recorded lower seroprevalence in bulls (8.25%) of Gujarat with 16.31% in cattle and 0.99% in buffalo bulls.

Comparative efficacy of diagnostic tests revealed similarity with the observations of Varasada (2003) who reported that seropositivity by Indirect ELISA is highest (24.13%) as compared to RBPT (13.79%) and STAT (13.79%) in bulls. The present findings are in agreement to those observed by Rao *et al.* (1999), Barbuddhe *et al.* (2004), and Bhattacharya *et al*. (2005) in cattle and buffaloes. This might have been due to the ability of ELISA to detect all types of immunoglobulins.

Regarding sensitivity and specificity of the diagnostic tests, present findings corroborated the reports of Kanani *et al.* (2007), who found higher sensitivity of STAT (62.5%) than RBPT (50%) while slightly higher specificity of RBPT (98.31%) over STAT (97.75%). On the contrary, Gall and Nielsen (2004) reported higher sensitivity of RBPT but higher specificity of STAT.

Therapeutic study revealed similar observations with that made by Kumar *et al*. (2005) who reported that treatment with longacting oxytetracycline and streptomycin combination resulted in (1 to 2 dilution) decrease in serotitre. Hall and Manion (1970) also reported that *Brucella* organisms are sensitive against tetracycline and streptomycin. On the contrary, Nicoletti *et al.* (1985) reported that titre test was of limited value in short term evaluation of therapeutic regimens.

CONCLUSION

The present study concludes that the ELISA found to be most sensitive test for screening animals against Brucellosis. The therapeutic study of brucella infected animals revealed that long acting Oxytetracycline and Streptomycin combination had a significant decrease in the antibody titre on the 30th day of post treatment.

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