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Seroprevalence of Bovine Brucellosis in Different Agro-Climatic Regions of Punjab

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ABSTRACT

This cross sectional study was designed to determine the current status and prevalence of brucellosis in cattle and buffaloes. A total of 1220 animals (895 cows, 325 buffaloes) from the five agro-climatic regions of Punjab were screened for brucellosis using rose bengal plate agglutination (RBPT) and microtiter plate agglutination test (MAT), from April 2012 to May 2013. In the study population, an overall seroprevalence of brucellosis was observed as 27.95 and 18.11% by RBPT and MAT, respectively. Statistically significant ($p<0.01$) differences in prevalence of brucellosis among cattle and buffaloes were 23.01% (206/895), 18.21% (163/895) and 30.99% (84/325), 19.92% (54/325) by RBPT and MAT, respectively. Among the two tests applied in this present study, RBPT was found to be more sensitive than MAT.

Key words: Brucellosis, cattle, buffaloes, RBPT, MAT

INTRODUCTION

Brucellosis is considers to be a neglected zoonoses because, despite its widespread distribution and effects on multiple species, it is not prioritized by national and international health systems (WHO., 2006). The disease is caused by gram negative coccobacilli of genus *Brucella* (Gupta *et al*., 2014) which has been classified into different species on the basis of preference of each species to its natural host that also serves its reserviour. *Brucella* organisms are transmitted from the infected animals to man by ingestion of unpasteurized milk and milk products by contact with infected animals or their discharges or by inhalation of aerosols containing *Brucella* organisms (Refai, 2002). Bovine brucellosis is mainly caused by the *Brucella abortus* and less frequently by *B. melitensis*. The disease is endemic in most part of the world particularly in developing countries including India and causes a variety of reproductive disorders viz. infertility, late term abortion, retention of placenta and endometritis etc (Kumar *et al*., 2009; Dhama *et al*., 2013). It results into a heavy economic loss to the farmer, a leading impediment to the profitability (Tiwari *et al*., 2013). In India, the disease is appears to be on the increase in recent times, perhaps due to the changing husbandary practices from traditional to modern livestock rearing, coupled with stocking of more number of animals per unit are have resulted in spatial clustering of both infection and the disease (Renukaradhya *et al*., 2002). Moreover risk pattern in India such as unrestricted trade and movement of animals, use of local cattle yards and fairs for trading, sending dry animals back to villages for maintenance, use of semen from unscreened bulls for artificial insemination and poor

farm hygiene contribute to the spread and transmission of the infection (Smits and Kadri, 2005). The definate diagnosis of brucellosis is made primarily either by isolation of the *Brucella* or by a combination of the serological tests and clinical findings (Poester *et al*., 2010). Numerous serological tests have been used for the diagnosis of bovine brucellosis as a screening or confirmatory test. Rose Bengal Plate Test (RBPT) based on agglutination of coloured particulate antigen (killed *Brucella* organisms) by the antibodies present in the patient's serum is the most common test for brucellosis in the field condition. The present study was therefore, carried out to study the current status and epidemiology of brucellosis in cattle and buffaloes in five agro-climatic regions of Punjab using a very simple test i.e., rose bengal plate agglutination test (RBPT) and microtiter plate agglutination test (MAT).

MATERIALS AND METHODS

Sample collection: A total of 1220 (895 cattle and 325 buffaloes) animals from the five different agro-climatic regions of Punjab was included in the study. Blood samples were aseptically collected from all the animals by jugular vein. About 5-10 mL of blood was collected in glass tubes without any anticoagulant. The blood samples were kept on ice immediately and until transported to laboratory. Serum was separated from clotted blood by centrifugation at 3000 rpm for 5 min and stored at -20°C till further use. Sera sample were subjected to analysis by Rose Bengal Plate agglutination Test (RBPT) and then Microtiter Plate agglutination Test (MAT).

Rose Bengal plate agglutination test (RBPT): The RBPT was performed as per the protocol (Alton *et al*., 1975). In brief, serum samples and RBPT antigen procured from Punjab State Veterinary Vaccine Institute, Ludhiana were brought at room temperature. One drop (0.03 mL) of serum and one drop of antigen was taken on a clean, dry and non-greasy glass slide and were mixed thoroughly to observe agglutination formed within four min.

Microtiter plate agglutination test (MAT): The MAT was performed as per the protocol (Alton *et al*., 1975) on 96 well round bottom microtiter plate using *Brucella* plain antigen procured from Punjab State Veterinary Vaccine Institute, Ludhiana. In brief, serum was serially diluted by adding 80 μL of phenol saline and 20 μL of serum in the first well. After mixing, 50 μL was transferred from the 1st well to the 2nd well having 50 μL of phenol saline. The process was continued up to the 11th well and finally 50 μL was discarded from the last well. Then, 50 μL of antigen was added to all the wells and mixed thoroughly. A control tube indicating 50% agglutination was set up adding 50 μL of antigen and phenol saline. The plate was incubated at 37°C for 20 h to judge the agglutination on the basis of opacity of the supernatant fluid. The highest serum dilution showing 50% or more agglutination (50% clearing) was considered as the titre of the serum and was doubled to get International Unit (IU) per mililiter of serum. Positive and negative controls were used in each series of test runs. A serum titer of 80 IU or above was considered as positive whereas, 40 IU as doubtful and less than 40 IU as negative for MAT.

Statistical analysis: Data from laboratory test and signalment of each animal were stored in excel spread sheet. The data were analyzed using Chi-Square (χ^2) test.

RESULTS

Seroprevalence of brucellosis in the five agro-climatic regions of Punjab state was estimated on the basis of results obtained by RBPT and MAT. An overall seroprevalence of 27.95% (341/1220)

Table 1: Overall seroprevalence in different agroclimatic regions of Punjab

			RBPT		MAT	
Agroclimatic regions	Districts	No. of animals tested	No. of sample positive	Percentage	No. of sample positive	Percentage
Submountain undulating	Gurdaspur	123	28	22.76	15	12.19
	Hoshiarpur	42	12	28.57	6	14.28
Undulating plain	Ropar	30	14	46.66	11	36.66
	Nowanshahr	64	11	48.43	6	9.375
Central plain	Amritsar	55	25	45.45	21	38.18
	Jalandhar	70	23	32.85	20	28.57
	Ludhiana	233	47	20.17	37	15.87
	Sangrur	62	31	50.00	30	48.38
	Patiala	160	50	31.25	26	16.25
Western plain	Ferozepur	53	22	41.50	20	37.73
	Faridkot	71	18	25.35	11	15.49
Western zone	Moga	120	16	13.33	11	9.16
	Bathinda	44	13	29.54	6	13.63
	Muktsar	45	12	26.66	3	6.66
	Barnala	48	13	27.08	4	8.33
Total		1220	341	27.95	221	18.11
Chi-square between agroclimatic regions (p<0.01)			100.959*		108.145*	
Chi-square between tests (p<0.01)			33.291*			

RBPT: Rose bengal agglutination test, MAT: Microtiter plate agglutination test

and 18.11% (221/1220) of brucellosis was observed in the study population by RBPT and MAT as a confirmatory test in this work (Table 1). Species, age and history of abortion were the host factors studied.

The prevalence of brucellosis among cattle and buffaloes were 37.45% (339/905), 31.60% (286/905) and 35.22% (93/264), 27.27% (72/264)) by RBPT and MAT, respectively and the differences observed were statistically significant (p<0.01) (Table 2 and 3).

With regards to age, the prevalence of brucellosis was higher in both cattle and buffaloes of age groups 3-4 years (58.70 and 51.01%),(64.93 and 58.44%) followed by animals in the age groups of 5-6 years (51.81 and 43.00%),(57.69 and 38.46%), 7-8 years (21.10 and 19.26%),(11.11 and 5.55%) and least in animals of 7 months 2 years (19.94 and 15.73%),(9.09 and 5.05%) of age group by RBPT and MAT, respectively (Table 4). The differences in the prevalence of the disease among these four age groups were statistically significant (p<0.01) with animals in the age group of 3-4 years being the most susceptible.

With regard to history of abortion animals, up to two years of age were excluded from the data so as to avoid bias. Out of the remaining animals, 17.42% (107/614) were having history of abortion. Of these aborted animals, 81.36% (87/107), 57.94% (62/107) were sero-positive by RBPT and MAT. The seroprevalence of brucellosis was significantly (p<0.01) higher in animals with a history of abortion 81.36% (87/107), 57.94% (62/107) than in those without such histories as 21.80% (121/555) and 19.63% (109/555) by both RBPT and MAT, respectively (Table 5). Out of the total 107 cases of abortion recorded in cattle and buffaloes, 83 cases of abortions occurred in the third trimester, 22 in the second and 1 in the first trimester of gestation. The seroprevalence of brucellosis was (50.00 and 0.00%), (72.72 and 45.45%), (69.87 and 50.60%) by RBPT and MAT in animals with history of abortion in first, second and third trimesters of gestation, respectively the differences were statistically significant (p<0.01). Out of 97 aborted animals, 55.67% (54) had history of retention of placenta and of these 32 were cows and 22 were buffaloes (Table 6).

DISCUSSIONS

Brucellosis remains one of the major public health concerns throughout the developing world (Karthik *et al*., 2013). Seroprevalence of bovine brucellosis have been assessed at different times

Table 2: Agroclimatic regions based distribution of brucellosis antibodies in cattle

			RBPT		MAT	
Agroclimatic regions	Districts	No. of animals tested	No. of sample positive	Percentage	No. of sample positive	Percentage
Submountain undulating	Gurdaspur	114	21	18.42	8	7.01
	Hoshiarpur	10	2	20.00	0	0
Undulating plain	Ropar	8	1	12.50	1	12.5
	Nowanshahr	17	2	11.76	1	5.88
Central plain	Amritsar	44	19	43.18	17	38.63
	Jalandhar	60	21	35.00	19	31.66
	Ludhiana	179	32	17.87	27	15.08
	Sangrur	51	30	58.82	29	56.86
	Patiala	148	48	32.43	24	16.21
Western plain	Ferozepur	43	17	39.53	15	34.88
	Faridkot	20	6	30.00	4	20.00
Western zone	Moga	107	12	11.21	8	7.47
	Bathinda	31	8	25.80	4	12.90
	Muktsar	35	10	28.57	3	8.57
	Barnala	28	7	25.00	3	10.71
Total		895	206	23.01	163	18.21
Chi-square between agroclimatic regions (p<0.01)			70.177*		103.081*	
Chi-square between tests (p<0.01)			6.312*			

RBPT: Rose bengal agglutination test, MAT: Microtiter plate agglutination test

Table 3: Agroclimatic regions based distribution of brucellosis antibodies in buffaloes

			RBPT		MAT	
Agroclimatic regions	Districts	No. of animals tested	No. of sample positive	Percentage	No. of sample positive	Percentage
Submountain undulating	Gurdaspur	9	7	77.77	7	77.77
	Hoshiarpur	32	10	31.25	6	18.75
Undulating plain	Ropar	22	12	54.54	10	45.45
	Nowanshahr	47	10	21.27	5	10.63
Central plain	Amritsar	11	6	54.54	4	36.36
	Jalandhar	10	2	20.00	1	10.00
	Ludhiana	54	15	27.27	10	18.51
	Sangrur	11	1	9.09	1	9.09
	Patiala	12	2	16.66	2	16.66
Western plain	Ferozepur	10	5	50.00	5	50.00
	Faridkot	51	12	23.52	7	3.72
Western zone	Moga	13	4	30.76	3	23.07
	Bathinda	13	5	38.46	2	15.38
	Muktsar	10	2	20.00	0	0.00
	Barnala	20	6	30.00	1	5.00
Total			325	99	30.46	6419.69
Chi-square between agroclimatic regions (p<0.01)			28.451*		66.762*	
Chi-square between tests (p<0.01)			10.031*			

RBPT: Rose bengal agglutination test, MAT: Microtiter plate agglutination test

Table 4: Age wise prevalence of brucellosis

Species	Age group	Animal tested	RBPT	Positive by RBPT (%)	MAT	Positive by MAT (%)
Cattle	7M-2Y	346	71	20.05	56	16.18
	3-4Y	247	145	58.70	126	51.01
	5-6Y	193	100	51.81	83	43.00
	7-8Y	109	23	21.10	21	19.26
Total		895	339	37.45	286	31.60
χ^2 test within the age group (p<0.01)		118.800*			173.072*	
Buffaloes	7M-2Y	99	9	9.09	5	5.05
	3-4Y	138	40	28.98	30	21.73
	5-6Y	52	10	34.61	14	26.92
	7-8Y	36	4	11.11	2	5.55
Total		325	63	35.22	51	27.27
χ^2 test within the age group (p<0.01)		20.949*			20.042*	

RBPT: Rose bengal agglutination test, MAT: Microtiter plate agglutination test

Table 5: Brucellosis in aborted animals and retention of placenta

	Cattle			Buffaloes		
Species	Total	RBPT	MAT	Total	RBPT	MAT
Abortion	72	54	42	25	22	20
Retention of placenta	32	17	14	22	16	12

RBPT: Rose bengal agglutination test, MAT: Microtiter plate agglutination test

Table 6: Trimester of pregnancy at the time of abortion

Term of pregnancy	Aborted	RBPT positive	MAT positive
1st	2	1	0
2nd	22	16	10
3rd	83	58	42
Chi-square test between terms (p<0.01)		0.460*	2.111*

RBPT: Rose bengal agglutination test, MAT: Microtiter plate agglutination test

from different regions of the country (Kumar *et al*., 2009; Chand and Chhabra, 2013; Patel *et al*., 2014). An overall seroprevalence of 27.95% (341/1220) and 18.11% (221/1220) of brucellosis was observed in different agro-climatic regions of Punjab by RBPT and MAT as a confirmatory test in this work which is comparable to 37.38 and 36.45% (Barbuddhe *et al*., 2004), 58.9 and 55.2% (Genc *et al*., 2005) were positive for *Brucella* antibodies by RBPT and STAT, respectively in Goa. In Punjab, prevalence rates among buffaloes and cattle in 2005 were 13.4 and 9.9%, respectively by Avidin biotin ELISA (Dhand *et al*., 2005) whereas in 2008, it's being increased upto 16.41 and 20.67%, respectively and the overall prevalence rate of brucellosis was found to 18.26% by milk ELISA (Aulakh *et al*., 2008) whereas herd prevalence was 72.72% in Punjab based on the eight districts (Chand and Chhabra, 2013). The difference in seroprevalence obtained by different workers may be due to sensitivities and specificities of the different diagnostic methods used among researchers.

The prevalence of brucellosis among cattle and buffaloes were 23.01% (206/895), 18.21% (163/895) and 30.99% (84/325), 19.92% (54/325) by RBPT and MAT, respectively and the differences observed were statistically significant (p<0.01). These findings were in concurrence with that of (Chand and Chhabra, 2013), but differed from that of (Saini *et al.,* 1992), who reported higher disease prevalence in buffaloes. Dhand *et al*. (2005) reported that cattle and buffaloes are equally susceptible to the *Brucella* infection. Cross bred cattle are more susceptible to stress conditions than buffaloes, the genetic differences especially in innate immune system may also be the possible reason for variation in seroprevalence between the two species.

The higher seroprevalence rate from RBPT in this study may be due to the relatively low specificity and very high sensitivity of the test (Smits and Kadri, 2005). Apparently it could be as a result of cross reaction of smooth lipopolysaccharide (SLPS) with other gram negative organisms. However, no single test provides 100% sensitivity and specificity. To compare the sensitivity and specificity of RBPT, STAT and AB-ELISA, a serological survey was performed (Singh *et al*., 2004) in 6 organized dairy farms in Punjab. The study revealed that the sensitivity of RBPT (88.46%) was higher when compared with STAT (46.15%), while specificity of STAT (98.31%) was slightly higher than RBPT (97.75%). Despite of these limitations, the RBPT is used as initial screening test in the livestock. The lesser incidence with the MAT was indicative of its very specific than RBPT.

With respect to age, the seroprevalence of brucellosis was higher in animals of age group 3-4 years followed by animals in the age group of 5-6 years and then in 7 M 2 year and least in animals of 7-8 years of age and the differences observed were statistically significant. The results of the present study showed that animals of 3-4 years of age are more likely to become sero positive to brucellosis. These findings are in agreement with the results of Aulakh *et al*. (2008),

Abubakar *et al*. (2010) and Mohammed *et al*. (2011) who reported that, incidence is higher in sexually matured animals. The animals of these age groups are actively involved in breeding. Lower prevalence of brucellosis in young ones could be due to resistance of young animals to infection (Nicoletti, 1980). Dhand *et al*. (2005) suggested that with passage of time animals are more likely to be exposed to the bacteria and contract the disease. However, Amin *et al*. (2005) reported that, high prevalence of brucellosis among old animals might be related to maturity with advancing age, thereby the organism may have propagated to remain as latent infection or it may cause disease.

Outbreaks of bovine brucellosis are associated with abortion from 5th to 8th month of gestation, retention of placenta, production of weak new born calves and infertility in animals (OIE., 2009). With respect to abortion history, female animals greater than two years of age were included in the analysis to avoid bias. There were 11.13% of animals (91/817) had a history of abortion and of these 75.82% (69/91) were sero-positive. Statistically significant differences in seroprevalence between animals with history of abortion and those without such histories were observed, these findings were in concurrence with that of Sandhu *et al*. (2001), who reported a higher seroprevalence of brucellosis in animals with history of abortion. Though other infectious and non-infectious causes prevalent in the study area could also have contributed to abortion, however the results of the present study indicated that brucellosis was a major cause of abortion in dairy animals. Most of these abortions took place in the last trimester of gestation, followed by second trimester and least in first trimester.

A higher seroprevalence of brucellosis was revealed in the present study, which is a serious public and animal health threat. Therefore, a constant monitoring system needs to be in place to study the changes in the disease dynamics so that the control strategies can be manipulated to bring down the incidence and prevalence of the disease to a justifiable level. Prevalence was statistically different in cattle and buffaloes, but both species need to be taken into consideration while implementing the control programmes.

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