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Seroprevalence of brucellosis among bovine populations of southwestern Punjab, India

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Abstract

Brucellosis is an economically important zoonotic disease of dairy industry. This study was designed to assess individual animal and herd level seroprevalence of bovine brucellosis in dairy herds located in Southwestern Punjab, India. A total of 898 bovine [cattle (n= 623); buffaloes (n= 275)] serum samples from six districts [Bathinda (n= 769), Barnala (n= 100), Muktsar (n= 22), Mansa (n= 03), Faridkot (n= 03) and Moga (n= 01)] of Southwestern Punjab were received for diagnosis of brucellosis. The samples were belonging to 260 dairy herds and animals were suffering from reproductive disorders such as abortion, infertility, repeat breeding, hygroma etc. Initially, the Rose Bengal plate agglutination test (RBPT) was performed to screen serum samples for qualitative detection of anti-Brucella antibodies. In RBPT, 31.18 per cent (280/898) of serum samples were positive for brucellosis. Among the 260 herds, 98 (37.69%) herds were positive for the disease. The RBPT-positive samples were further processed for quantitative detection of antibody titre using standard plate agglutination test (STAT). In STAT, 72.50 per cent (203/280) of RBPT-positive samples were positive. The highest prevalence was reported in Mansa (66.67%), followed by Bathinda (32.89%), Barnala (23%), and Muktsar (9.1%). None of the samples from Faridkot and Moga districts tested positive. Three host factors, viz, species, sex, and age, were included in the analysis of disease status. There was no statistical variation in the disease prevalence among cattle and buffaloes, and males and females. However, the prevalence was higher among animals aged more than one year (30.21-34.24%) than those aged less than one year (22.22%). Further studies need to be conducted with large sample size to determine the true burden of disease in Punjab, especially in the Southwestern region.

Keywords: *Bovine Brucellosis, Punjab, Seroprevalence, Zoonosis*

Brucellosis, considered to be an economically important disease affecting dairy industry, is responsible for causation of infertility, abortion, weak offspring, retained placenta, endometritis and reduced production in animals (Franc *et al*., 2018; Singh *et al*., 2020). The disease is highly endemic in different states of the country (Chand and Chhabra, 2013; Shome *et al*., 2019). In India, the annual economic losses due to brucellosis in livestock have been reported to be US$ 3.4 billion, with loss of cattle and buffalo accounting to 95.6 per cent of the total losses (Singh *et al*., 2015). The reproductive systems of sexually mature animals act as sites of predilection and multiplication of pathogens. However, in lactating animals, the pathogen is localised in the mammary tissues and frequently excreted in milk (Kushwaha *et*

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al., 2016). The disease is transmitted in humans while bare handling placenta, fetal fluid, vaginal discharge and drinking raw milk of brucellosis-infected animals (Bano and Lone, 2015; Muthiah *et al*., 2024). In humans, the disease manifests as acute, subacute and chronic febrile forms, with night sweats, joint pain and weakness (Mantur *et al*., 2007; Proch *et al*., 2018). Unrestricted movement of infected animals within and between states, ban on cattle slaughter due to religious sentiments, impractical testing and segregation of infected animals by livestock owners, intensive dairy farming and selling of infected animals in animal fares are responsible for spreading and maintaining the infection in the country (Chand and Chhabra, 2013). Isolation of pathogen is considered to be most confirmatory test for disease diagnosis. However, it is time-consuming and requires biosafety level 3 (BSL-3) facilities to handle suspected clinical samples (Godfroid *et al*., 2010; OIE, 2013; Kaynak-Onurdag *et al*., 2016). For molecular detection of pathogen, polymerase chain reaction (PCR)-based assays are commonly performed, which are highly sensitive and specific, for rapid and accurate detection of pathogen (Navarro *et al*., 2004; Sundar *et al*., 2015). However, it requires sophisticated laboratory facilities and skilled man power, which hinders its wider application in resource limited laboratories (Ahmed *et al*., 2015). Serological tests such as, Rose Bengal Plate Agglutination Test (RBPT), Standard Tube Agglutination Test (STAT) and Enzyme-linked Immunosorbent Assay (ELISA), are most commonly used for detection of anti-*Brucella* antibodies from serum samples of animals and humans. These tests are fast, inexpensive and sensitive, but not highly specific (Kushwaha *et al*., 2016). The RBPT and STAT are considered to be "conventional" or "classical" tests for diagnosis of brucellosis, with RBPT being used as a primary screening test for rapid detection of disease at the individual animal level, even if the antibody titer is low (OIE, 2013). It remains an adequate assay for detecting infected herds and guarantees the absence of infection in brucellosis-free herds (OIE, 2018). In a study to evaluate the diagnostic efficacy of lateral flow assay, indirect ELISA (iELISA) and RBPT for the detection of brucellosis in cattle, RBPT showed the highest diagnostic sensitivity (98.9%) and specificity (100%) (Gusi *et al*., 2019). The STAT is a simpler and cheaper test, mainly used for the diagnosis of acute cases of brucellosis (Barbuddhe *et al*., 2020; Qureshi *et al*., 2023). It is commonly used for the quantification of anti-*Brucella* antibodies; however, its application is limited in chronically infected herds due to dominance of non-agglutinating IgG in such cases (Yohannes *et al*., 2012; Ducrotoy *et al*., 2018). Previous studies have demonstrated higher sensitivity (Se) and specificity (Sp) of RBPT (Sp: 100%; Sp: 100%) when compared to STAT (Se: 81.5%; Sp: 98.9%) (Díaz-Aparicio *et al*., 1993; McGiven *et al*., 2003; Ducrotoy *et al*., 2018). The present study was designed for detection of individual animal and herd level seroprevalence of brucellosis in bovine herds located in Southwestern region of Punjab using RBPT and STAT.

Materials and methods

Study design

In this cross sectional study, a total of 898 blood samples from bovines [cattle (n= 623); buffaloes (n= 275)] of 260 dairy herds, having history of reproductive disorders such as abortion, infertility, repeat breeding, hygroma etc. were received in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Rampura Phul (GADVASU) during May, 2022 to April, 2024 from six districts [Bathinda (n= 769), Barnala (n= 100), Muktsar (n= 22), Mansa (n= 03), Faridkot (n= 03) and Moga (n= 01)] of Punjab for diagnosis of brucellosis. The samples were collected in a 5 mL capacity clot activator tube for serum separation. After receiving the samples, the tubes were kept at room temperature in an undisturbed slanting position for 30-60 min, then it was centrifuged at 2500×g for 10 min. The serum was separated and initially processed for detection of brucellosis using RBPT and later quantification of anti-*Brucella* antibodies using STAT.

Serological screening for detection of brucellosis

Rose Bengal Plate Agglutination Test (RBPT): An equal volume (30 µL) each of *Brucella abortus* S99 colored antigen (procured from Punjab Veterinary Vaccine Institute, Ludhiana) and test serum was kept on grease free glass slide, mixed it and rotated close wise and anti-clock wise direction up to four minutes. The mixture showing agglutination was considered positive and no agglutination was considered negative for brucellosis.

Standard Tube Agglutination test (STAT): To carry out this test**,** serial dilution of test serum was performed in phenol saline, followed by adding an equal quantity (0.5 ml) of *Brucella abortus* S99 plain antigen (procured from Punjab Veterinary Vaccine Institute, Ludhiana) into each tube (final dilution 1:10-1:160). After proper mixing, the tubes were incubated overnight at 37°C. The tube showing 50 per cent agglutination was selected for calculation of antibodies titre. The titre value of ≥1:40 (80 IU) was considered as a positive sample.

Statistical analysis

The data obtained from RBPT and STAT was statistically analysed using Epitools Epidemiological Calculators, considering 95 per cent confidence interval (Rogan and Gladen, 1978).

Results and discussion

A total of 898 blood samples of bovines [cattle (n= 623); buffaloes (n= 275)] having history of reproductive disorders were received from six districts [Bathinda (n= 769), Barnala (n= 100), Muktsar (n= 22), Mansa (n= 03), Faridkot (n= 03) and Moga (n= 01)] of Punjab, India for diagnosis of brucellosis (Fig. 1). The samples belonged to

260 bovine herds of Southwestern, Punjab. After separation of serum from collected blood, RBPT was performed for qualitative detection of anti-*Brucella* antibodies. In RBPT, 31.18 per cent (280/898; 95% CI; 28.24-34.28) of serum samples were found positive for brucellosis (Table 1). The herd prevalence was 37.69 per cent (98/260; 95% CI; 32.02-43.72) (Table 2). The RBPT-positive samples were further processed for the quantitative detection of anti-*Brucella* antibodies using STAT. In STAT, 72.50 per cent (203/280; 95% CI; 66.99-77.40) of RBPT-positive serum samples were found positive (Table 1). The antibody titers of the STAT-positive samples are tabulated in Table 3.

Brucellosis is an economically important zoonotic disease of livestock. The disease is endemic in several developing countries, including India (Karthik *et al*., 2013; Shome *et al*., 2019). The government of India has taken initiative for control of brucellosis by launching National Animal Disease Control Programme (NADCP) in 2019. Still, the disease remains a major problem in livestock sector as it causes reproductive disorders in animals such as abortion, infertility, repeat breeding, hygroma and reduced milk production (Shome *et al*., 2019; Sharma *et al*., 2023). Proper surveillance and vaccination of female calves of 4-8 months of age are the two most effective approaches for controlling the disease in the country. However, this task is very difficult because of the limited availability of vaccines, manpower and laboratory services compared with the huge bovine population of the country (Shome *et al*., 2019).

Figure 1: Seroprevalence study of bovine brucellosis in Southwestern Punjab, India

The present study shows a high prevalence (31.18%) of bovine brucellosis in Southwestern region of Punjab, India. The results suggest that bovine brucellosis is highly endemic in this region, with a herd prevalence of 37.69 per cent. Previous studies have reported varying levels of seroprevalence of bovine brucellosis in the state, ranging from 9.67-34.15 per cent (Sandhu *et al*.,

Table 1: Individual animal level seroprevalence of bovine brucellosis in Southwestern Punjab

Variables	No. of samples tested	RBPT		STAT of RBPT-positive samples	
		No. of samples positive (%)	95% CI	No. of samples positive (%)	95% CI
Districts					
Bathinda	769	253 (32.89)	29.67-36.30	186/253 (73.51)	67.76-78.57
Barnala	100	23 (23)	15.84-32.15	15/23 (65.22)	44.89-81.19
Muktsar	22	2 (9.1)	2.53-27.81	0/2 (0)	0-65.76
Mansa	03	2 (66.67)	20.77-93.85	2/2 (100)	34.25-100
Faridkot	03	0 (0)	0-56.15	0 (0)	0
Moga	01	0 (0)	0	0 (0)	0
Species					
Cattle	623	194 (31.14)	27.63-34.88	144/194 (74.23)	67.64-79.87
Buffalo	275	86 (31.27)	26.08-36.98	59/86 (68.60)	58.18-77.44
Sex					
Female	867	269 (31.03)	28.04-34.18	197/269 (73.23)	67.64-78.17
Male	31	11 (35.48)	21.12-53.05	06/11 (54.55)	28.01-78.73
Age					
< 1 year	09	02 (22.22)	6.32-54.74	0/02 (0)	0-65.76
1-3 years	219	75 (34.24)	28.28-40.75	51/75 (68.0)	56.79-77.46
3-6 years	523	158 (30.21)	26.43-34.28	119/158 (75.32)	68.05-8139
>6 years	148	45 (30.40)	23.73-38.48	33/45 (73.33)	58.96-84.04

Table 2: Herd seroprevalence of bovine brucellosis in Southwestern Punjab

Districts	Number of animal farms tested	No. of infected animal farms	Herd prevalence (%)	95% CI
Bathinda	228	87	38.16	32.10-44.61
Barnala	22	7	31.82	16.36-52.68
Muktsar	3	2	66.67	20.77-93.85
Mansa	3	2	66.67	20.77-93.85
Faridkot	3	0	0	0
Moga	1	0	0	0
Total	**260**	**98**	**37.69**	**32.02-43.72**

Table 3: Titer values of STAT positive bovine serum samples (n = 203)

Variables	No. of STAT positive samples	Titre value		
		80 IU	160 IU	≥320 IU
Districts				
Bathinda	183	75	49	59
Barnala	15	3	5	7
Muktsar	0	0	0	0
Mansa	5	3	1	1
Faridkot	0	0	0	0
Moga	0	0	0	0
Species				
Cattle	144	59	39	46
Buffalo	59	22	16	21
Sex				
Female	197	79	53	65
Male	06	2	2	2
Age				
< 1 year	0	0	0	0
1-3 years	51	18	15	18
3-6 years	119	49	34	36
>6 years	33	14	6	13

2001; Dhand *et al*., 2005; Aulakh *et al*., 2008; Yohannes *et al*., 2012; Chand and Chhabra, 2013; Islam *et al*., 2013; Holt *et al*., 2021; Sharma *et al*., 2023). The differences in seroprevalence among these studies may be due to the different survey techniques employed by researchers and different diagnostic tests employed for the screening of samples (Dhand *et al*., 2005; Aulakh *et al*., 2008). In this study, approximately one-third of the dairy farms (98/260) had at least one animal seropositive for the disease. The findings of the present study are consistent with those of an earlier study on the herd prevalence of disease (Holt *et al*., 2021). The reason of high prevalence of brucellosis observed in this study might be due to the fact that all animals tested in the study were suffering from at least one reproductive disorder, such as abortion, infertility, repeat breeding and hygroma, which is a characteristic clinical symptom of brucellosis in animals. Earlier researchers also reported the high prevalence of brucellosis in animals having reproductive disorders, such as abortion and infertility, as compared to healthy animals (Dhand *et al*., 2005; Islam *et al*., 2013).

Among districts, the highest prevalence was reported from Mansa (66.67%) followed by Bathinda (32.89%), Barnala (23%) and Muktsar (9.1%). This high prevalence rate may be due to the greater movement of brucellosis-infected animals in these districts. Lack of awareness of the disease among animal handlers of dairy farms located in these districts may also be a possible reason. The high prevalence of disease in Mansa and Bathinda compared to Barnala and Muktsar may be because former two districts are located in the same agro-climatic zone of Punjab, i.e., in the western region whereas the latter two in Western plane region of Punjab. Previous studies also support the agro-climatic region-wise variation in disease prevalence in the state (Sandhu *et al*., 2001; Dhand *et al*., 2005; Aulakh *et al*., 2008). As cattle slaughter is banned in the state, livestock owners mostly sell disease-positive animals in animal fares, which results in transmission of the infection to other farms in same or other districts. Farmers usually do not test animals for brucellosis prior to purchase, which leads to the entry of the disease in brucellosis free farms (Aulakh *et al*., 2008; Chand and Chhabra, 2013).

Three host factors i.e., species, sex and age were included to analyse the status of disease. In species-wise prevalence, a slightly higher prevalence was reported in buffaloes (31.27%) than cattle (31.14%). However, the differences were found to be non-significant. Earlier studies also reported high prevalence of brucellosis in buffaloes as compared to cattle (Sharma and Saini 1995; Dhand *et al*., 2005; Basit *et al*., 2015).

Among sex-wise prevalence, the disease was more prevalent in males (35.48%) than females (31.03%). The findings of the present study are concurrent with earlier study report in which higher prevalence of brucellosis was reported in males as compared to females in organized dairy farms located in western Uttar Pradesh (Kumar *et al*., 2016). However, most of the earlier studies reported a higher prevalence of disease in females as compared to males (Barman *et al*., 1989; Kushwaha *et al*., 2016; Gogoi *et al*., 2017). The higher prevalence of disease in males than in females in this study may be fallacious because of the small number of male serum samples (n=31) tested compared to female serum samples (n=867). Brucellosis-infected bulls in dairy farms may transmit the infection to female animals through natural breeding practices (Basit *et al*., 2015).

Age-wise, the highest prevalence of disease was reported in animals having age 1-3 years (34.24%), followed by animals of >6 years (30.61%), 3-6 years (30.21%), and <1 year (22.22%) of age. An earlier study reported a similar pattern of disease prevalence in different age groups of animals (Kumar *et al*., 2016). The prevalence of the disease in animals aged < 1 year may be

due to maternal transmission of infection to fetus in-utero, and feeding of milk from infected dams for a long period of time (Akhtar *et al*., 1990; Radostits *et al*., 2000). This may also be because of the continuous exposure of animals to contaminated dairy farm environment (Radostits *et al*., 2000; Chand and Chhabra, 2013). Earlier studies also reported a lower prevalence of disease in young animals as compared to adults (Paul, 1980; Dhand *et al*., 2005; Chand and Chhabra, 2013). Higher prevalence of disease in adults may be due to sexual maturity of animals with advancement of age and due to latent infections (Radostits *et al*., 2000; Kazi *et al*., 2005).

The important risk factors that are associated with disease in dairy farms are improper disposal of placenta and uterine discharges of brucellosis-infected animals, high animal density in farms leading to transmission of infection from infected to healthy animals, entry of newly purchased animals into herds without testing for brucellosis, ban on test and slaughter policy in India leading to transmission of infection from one area to another through the sale of infected animals and poor farm sanitary practices (Crawford *et al*., 1990; Chand and Chhabra, 2013).

Conclusion

The present study shows high prevalence of bovine brucellosis in some districts of Southwestern Punjab. The disease is almost equally prevalent in cattle and buffalo populations as well as in female and male animals. The prevalence of disease is found to be higher in animals more than one year of age than in animals less than one year of age. In this study, RBPT was used as a diagnostic test for brucellosis, followed by quantification of anti-*Brucella* antibodies using STAT. In future studies, more sensitive and specific tests, such as ELISA and PCR, should be included to determine the true prevalence of the disease in the region. In addition, for the effective control of disease, proper surveillance and vaccination of female calf of 4-8 months of age should be strictly followed.

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Conflicts of interest

The authors have declared that there is no conflict of interest.

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