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Prevalence of Exposure to *Brucella* Species in Lactating Cattle in the Ludhiana District of Punjab, India

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ABSTRACT

Brucellosis is a neglected endemic disease in Punjab and has a substantial impact on livestock and humans. A cross-sectional study was conducted to estimate the prevalence of *Brucella* species exposure in lactating cattle reared among dairy farmers in the Ludhiana district of Punjab, India. Blood samples were collected from 261 lactating animals (181 cattle and 80 buffalo) and were tested using Rose Bengal plate test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (i-ELISA). Information from the dairy farmers relating to animals such as breed of the cow, history of abortion, repeat breeding, retention of placenta was collected. An animal was considered overall seropositive based on a positive RBPT and a positive i-ELISA test. Of the 261 blood samples, 46 were positive using RBPT indicating an apparent sero-prevalence of 17.62%, and 58 were positive using i-ELISA reflecting an apparent sero-prevalence of 22.22%. The overall (animal positive in RBPT and a positive indirect ELISA test) apparent sero-prevalence was 15.71% (41 positive). The sero-prevalence was higher in cattle (18.23%) than in buffalo (10%). Regarding breed-wise, crossbreed cows showed slightly higher seropositivity (18.25%) than indigeneous cows (18.18%). Animals with a history of abortion, repeat breeding and retention of placenta (ROP) also showed seropositivity to brucellosis disease. More epidemiological investigations are required to generate data on the status of brucellosis in lactating cattle for the development of an extensive control program in India, including Punjab.

HIGHLIGHTS

- A Cross-sectional study focused on the prevalence of *Brucella* species in lactating cattle.
- The overall apparent sero-prevalence was 15.71%, higher in cattle than buffalo.
- Animals with a history of abortion, repeat breeding, and ROP also showed seropositivity to brucellosis.

Keywords: Brucellosis, i-ELISA, India, Lactating cattle, Prevalence, Punjab, RBPT

Brucellosis is an important disease affecting a wide range of domestic and wildlife mammals and caused by facultative, intracellular bacteria of the genus *Brucella* (Moreno, 2014). Brucellosis is considered the most devastating trans-boundary animal disease, causing major trade obstructions, and globally, it is the second most frequently reported zoonotic disease according to the World Organization for Animal Health (OIE) (OIE, 2016; WHO, 2015). *Brucella* infection in adult female cattle typically affects the reproductive system and can results in reduced fertility, placentitis, abortion (3rd trimester), premature delivery and significant decrease in milk production (Deka *et al.,* 2018). In humans, the disease is considered to be occupational and can be contracted from infected animals through contact with their aborted fetuses and related materials, ingestion of raw milk,or unpasteurized dairy products (OIE, 2016).

Bovine brucellosis is an endemic disease in India, including Punjab, and is frequently reported from marginal herds and organized farms, differing from area to area

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and farm to farm (Deka *et al.,* 2018; Renukaradhya *et al.,* 2002; Dhand *et al.,* 2005; Mantur and Amarnath, 2008). Brucellosis causes huge economic losses in India, both in terms of livestock and humans (Singh *et al.,* 2015; Singh *et al.,* 2018). Although, the disease is eradicated in many developed countries (Jarsen and Muller, 1982; Franc *et al.,* 2018), it remains a neglected disease in many developing countries, including India (Mantur and Amarnath, 2008).

Brucellosis can be screened and diagnosed by a number of serological tests, including the Rose Bengal Plate Test (RBPT), standard tube agglutination test (STAT), complement fixation test (CFT), milk ring test (MRT), enzyme-linked immune sorbent assay (ELISA), rapid slide agglutination test (RSAT), agar gel immunodiffusion (AGID), counter immunoelectrophorsis (CIE), and immunochromatographic test (ICT) (Sharma *et al.,* 2023). The most widely used screening test is the RBPT because of its high sensitivity (MacMillan, 1990), but it can also produce false negative results in certain circumstances. ELISA has been found to be of the same or greater sensitivity and specificity as RBPT and CFT (Blasco *et al.,* 1994), with the benefit of ease of performance and automation adaptability compared to CFT. It has been recommended to use a battery of serological tests for the diagnosis of brucellosis due to the differing sensitivities and specificities of individual tests (OIE, 2016).

The current study was, therefore, planned to estimate the prevalence of *Brucella* species exposure in lactating cattle in the Ludhiana district of Punjab, India. The study generates more epidemiological data on the status of bovine brucellosis in the lactating cattle for the development of an extensive control program in India, including Punjab.

MATERIALS AND METHODS

This cross-sectional study was approved by the Institutional Animal Ethics Committee, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab (GADVASU/2020/IAEC/55/16). Punjab (Latitude of 30.4N and Longitude 75.5E), an agrarian state, is home to 1135,771 in milk (lactating) livestock cattle (130,968 indigenous and 1004,803 exotic/crossbred cattle) and 1692,962 buffalo population (AHSD, 2019). The target population for this study was the lactating cattle of the Punjab state of India. The study population consisted of lactating cattle reared among dairy farmers in the Ludhiana district of Punjab, India.

Assuming a 21% sero-prevalence of brucellosis in the target population (Proch, 2016), the sample size was calculated to be 255 lactating cattle for prevalence estimation with a 95% confidence level, 5% absolute precision of error, and a design effect of 1.5 (Dhand and Khatkar, 2014). However, a total of 261 lactating cattle were sampled in order to account for samples that might later become unsuitable for testing.

A total of 261 lactating animals (181 cattle and 80 buffalo) were sampled from 10 randomly selected villages using a random number generator in the Ludhiana district of Punjab, India (https://www.calculator.net/randomnumber generator). The sample size per village was estimated using probability proportional to size sampling based on the number of animals in the selected villages. Households in these villages were randomly chosen using voter lists of villages to ensure the collection of a representative sample. Individuals who did not own the animals or belonged to the same family were substituted with the subsequent individuals in the voter list. Animals with in each household were selected using simple random sampling. Approximately 5 ml of blood was drawn aseptically from the jugular vein of each animal and left to clot. The clotted blood was centrifuged at 3000 rpm for 15 minutes in order to extract the serum. The resultant serum was aliquoted in screw-capped sterile vials and kept at -20°C until testing. Information from the dairy farmers relating to animals such as breed of the cow, history of abortion, repeat breeding, retention of placenta was collected.

All serum samples were subjected to a Rose Bengal Plate Test (RBPT), and indirect ELISA (i-ELISA). The RBPT antigen were obtained from the Punjab veterinary vaccine institute, Ludhiana, and the tests were done according to the World Organisation for Animal Health (OIE, 2016). Indirect ELISA was performed using a commercially available kit (Nova Tec Immunodiagnostica GmbH, Dietzenbach, Germany) in accordance with the manufacturer's instructions. The absorbance of all wells was measured at 450 nm, and results were interpreted using the formula given in the manufacturer's instructions in Nova Tec Units (NTU).

The cut-off value for indirect ELISA was calculated using the absorbance value of the standards provided along with the kit. Serum samples having NTU values of more than 11, 9-11, and less than 9 were categorized as positive,

equivocal/grey zone, and negative, respectively. The samples falling into equivocal/ grey zones were deemed inconclusive cases as antibodies against *Brucella* could not be clearly detected and needed to be retested. There were six samples that were inconclusive and declared negative. For overall positivity, the animal was determined to be seropositive by using a combination of tests in series, i.e. a positive RBPT and a positive Indirect ELISA test.

The apparent sero-prevalence estimates and their 95% confidence intervals were computed in Epitools (https://epitools.ausvet.com.au/trueprevalence; Brown *et al.,* 2001).

RESULTS AND DISCUSSION

Of the 261 blood samples, 46 were positive using RBPT, indicating an apparent sero-prevalence of 17.62%, and 58 were positive using i-ELISA, reflecting an apparent sero-prevalence of 22.22%. The overall (a positive RBPT and a positive indirect ELISA test) apparent sero-prevalence was 15.71% (41 positive) (Table 1).

The sero-prevalence was higher in cattle (18.23%) than in buffalo (10%). Regarding breed-wise, crossbreed cows showed slightly higher seropositivity (18.25%) than indigeneous cows (18.18%) (Table 2). There were 13 cattle and 3 buffalo having a history of abortion in all 261 animals sampled. Out of these, overall, 7 cows (53.85 %) and 3 buffalo (100%) were seropositive in both RBPT and i-ELISA (Table 2). Further, 5 cow and 4 buffalo were having history of ROP. Out of these, overall, 3 cows (60%) and 1 buffalo (25%) were seropositive in both RBPT and i-ELISA. Four cows were history of repeat breeding,and out of these, overall, 3 cows (75%) were seropositive in both RBPT and i-ELISA (Table 2).

Table 1: Apparent brucellosis sero-prevalence (%) estimated using different serological tests in 261 lactating animals in the Ludhiana district of Punjab, India

Diagnostic test	Number positive	Apparent prevalence	
		Prevalence (%)	95% CI
Rose bengal plate test	46	17.62	13.48-22.71
I-ELISA	58	22.22	17.60-27.65
Overall	41	15.71	11.80-20.62

Brucellosis causes significant economic losses for the livestock industry due to abortions, longer calving intervals, and decreased milk production (Singh *et al.,* 2015). However, many nations, including Britain, Sweden, Norway, Finland, Germany, Denmark, Belgium, Austria, Slovakia, Netherlands, Switzerland, Czech Republic, New Zealand, France, and Italy, have eradicated brucellosis in bovine populations using a test-and- slaughter approach (Jarsen and Muller, 1982; Franc *et al.,* 2018). Bovine brucellosis is endemic in India, and sero-prevalence in bovines is reported as around 12%, although highly varying between studies (Renukaradhya *et al.,* 2002; Deka *et al.,* 2018), differing from area to area and farm to farm (Mantur and Amarnath, 2008). Bovine brucellosis is also endemic in the Punjab, and several studies conducted in the state reported varying sero-prevalence estimates in farm animals ranging from 12.09% - 21.36% (Dhand *et*

Table 2: Results of serological tests for detection of *Brucella* spp. exposure in 261 lactating cattle and buffalo reared among dairy farmers in Ludhiana district of Punjab, India

Parameter	Category	Number tested	RBPT positive	i-ELISA positive	Overall positive
Species	Cattle	181	35	45	33
	Buffalo	80	11	13	8
Breed	Cross breed cattle	137	27	33	25
	Indigenous cattle	44	8	12	8
History of abortion	Cattle	13	7	7	7
	Buffalo	3	3	3	3
History of ROP	Cattle	5	3	4	3
	Buffalo	4	2	1	1
History of repeat breeding	Cattle	4	3	3	3
	Buffalo	0	0	0	0

al., 2005; UL-Islam *et al.,* 2013; Zadon *et al.,* 2015; UL-Islam *et al.,* 2018).

We reported 15.71% sero-prevalence of brucellosis in lactating cattle and buffalo populations reared among dairy farmers in Ludhiana district of Punjab, India. In comparison to our results, a very high proportion of 27.5% seropositivity in lactating cattle has been reported in Bangladesh (Islam *et al.,* 2021). The difference in proportion between India and Bangladesh could be attributed to several factors such as variation in the sampling and geographical range. Holt *et al.* (2021) found 15.1% sero-prevalence using milk samples from cattle and buffalo in rural Ludhiana district in Punjab, India. There are limited studies conducted to understand the epidemiology of brucellosis in lactating cattle in the Punjab.

Cattle are the common hosts for *Brucella abortus*, but the organism also causes disease in bison, water buffalo, elk, and camels and sporadically affects other species (Spickler, 2018). The current study also found brucellosis seropositivity in the buffalo population (10%). Although seropositivity in the buffalo population is lower than that in the cow population (18.25%). Previous studies also reported higher sero-prevalence in cow population than in the buffalo population (Holt *et al.,* 2021; Jaismon *et al.,* 2023). In the current study, cross-breed cows had a slightly higher sero-prevalence (18.25%) than indigeneous cattle (18.18%), and previous studies also reported high sero-prevalence in cross-breed cows than in indigeneous cows (Mangi *et al.,* 2015; Ndazigaruye *et al.,* 2018).

Brucella spp. in female cattle typically causes reproductive problems such as abortion, stillbirth, weak calves, retention of placenta, and repeat breeding (Smith and Kadri, 2005). This study found that the cattle with a history of abortion, repeat breeding, and retention of placenta showed seropositivity to brucellosis disease (Table 2). Previous studies also reported brucellosis seropositivity in animals with a history of abortion, repeat breeding, and retention of placenta (Shome *et al.,* 2023; Yanti *et al.,* 2021; Deka *et al.,* 2018; Gemma *et al.,* 2019; Deb Nath *et al.,* 2023).

Brucella species are shed in the milk of seropositive cattle (Capparelli *et al.,* 2009). The current study found 15.71% sero-prevalence of brucellosis in lactating cattle and buffalo populations reared among dairy farmers in the Ludhiana district of Punjab, India. Milk from these animals may be a probable risk factor for *Brucella* species infection in humans if consumed raw without boiling or pasteurization (Corbel, 2006).

This study had several strengths. The dairy farmers were randomly chosen from randomly selected villages using voter lists of villages in Ludhiana district of Punjab to ensure collection of representative samples. The sample size per village was estimated using probability proportional to size sampling based on the number of animals in the selected villages. Animals with in each dairy farmer were selected using simple random sampling. The study also had limitations. Due to limited resources and time, we could only sample dairy farmers residing in the Ludhiana district of Punjab. We also could not investigate risk factors associated with brucellosis in lactating cattle. Bovine brucellosis is endemic in India and more epidemiological investigations are required to generate more data on the status of bovine brucellosis in the lactating cattle for the development of an extensive control program in India, including Punjab.

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