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Seroprevalence and Associated Risk Factors of Bovine Brucellosis in Chhattisgarh Plains, India

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ABSTRACT

Background: Brucellosis is an important zoonotic disease, which mostly affects cattle causing abortion and infertility and thereby huge economical loss. Hence, it is highly essential to assess the status of the disease through proper diagnostic methods and accordingly prevention strategies may be taken for control and eradication.

Methods: A cross-sectional study was carried out in plain zone of Chhattisgarh to record the prevalence of bovine brucellosis and to analyse its associated risk factors during April' 2018-January' 2019. A total of 920 bovine sera samples were collected from non-vaccinated cross bred and non-descriptive cattle through multistage sampling. All the sera samples were screened with RBPT and indirect ELISA for detection of anti-*Brucella* antibody.

Result: The prevalence of brucellosis in cattle was found to be 21.3% (196/920) by i-ELISA and 14.24% (131/920) by RBPT. The risk association of several factors with the prevalence of brucellosis was figured through binary logistic regression analysis, where the risk of prevalence was higher among female cattle, in the Balod district and during winter season. The study indicated that sex, season and district has significant ($p \leq 0.05$) risk associated to the prevailing anti-*Brucella* antibody, which will further help in designing strategic prevention and control program of the disease.

Key words: Bovine brucellosis, Prevalence, Risk factors.

INTRODUCTION

Brucellosis is an important zoonotic disease next to rabies and is alluded to the group of important re-emerging neglected bacterial diseases of livestock, rendering a huge production loss and public health problems in India, despite several surveillance and control efforts (Boral *et al.* 2009; Kumar *et al*. 2010). The direct losses are due to reproductive disorders like infertility, retained placenta, abortion and endometritis (Lakshmikanth *et al*. 2021). The indirect losses are far more than direct losses. The infected/ carrier animals transmit infection to other animals and humans. The nomadic rearing of animals, sharing common pasture land and close interaction of humans had led to sharing infection in India. Several other factors like age, sex, breed, level of immunity and the environmental stress may attribute to probability of occurrence of infection in a herd.

Many researchers have worked on several host factors, management factors, farmer factors and agro-ecological factors and recorded their association with seropositivity (Deka *et al*. 2018). But very limited reports exist from an endemic country like India, briefing the risk association of different factors with prevalence. The previous study has focused on the influence of risk factors like age and sex to that of prevailing brucellosis in India (Chand and Chhabra, 2013). Hence, the present study was designed to figure out the prevalence of brucellosis among cattle in Chhattisgarh plains and its risk association to several factors (host and environment).

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MATERIALS AND METHODS

A total of 920 bovine sera samples were collected from the plain zone of Chhattisgarh during April' 2018 - January' 2019 and were stored at 4°C, in the Department of Veterinary Medicine, College of Veterinary Science and A.H., C.G.K.V., Anjora, Durg, Chhattisgarh. All of the sera were processed for indirect-Enzyme Linked Immunosorbent Assay (i-ELISA) and Rose Bengal Plate Agglutination Test (RBPT) at ICAR-IVRI, Izatnagar, Bareilly.

Random sampling

Sampling was done following a multistage sampling format. Firstly, five districts (Durg, Rajnandgaon, Raipur, Balod and Bemetara) of 16 present in plain region of Chhattisgarh, were selected for sampling. Subsequently from each district, one block was randomly selected (Durg, Rajnandgaon, Dharsiwa, Gunderdehi and Bemetara). In the third stage, identification of the farms (17 organized farms and 15 unorganized household rearing units) from each block was done. Lastly, the animal of either sex and different age groups were selected for collection of sera from each sector. The size of sample was estimated for both the sectors by using R statistical software R version with sampling book library (RV3.1.1, The R Foundation for Statistical Computing Platform) with an assumption of 10-20% for *B. abortus*, 95% confidence level, 80% power, 5% margin of error. Further, details related to the health condition and other management history of each animal was collected using a well-designed questionnaire (host and environmental risk factors).

Screening of bovine sera samples by RBPT

Firstly, all the 920 sera samples were screened by RBPT. 30 µl of "Rose Bengal Plate" antigen (Biological products, ICAR-IVRI) was placed on a clean glass plate and mixed with an equal volume of test sera sample. The glass plate was rocked at room temperature for few minutes and examined for agglutination reaction.

Detection of anti-*Brucella* antibody by i-ELISA

All the sera samples were examined for the presence of anti-*Brucella* antibody using i-ELISA which was developed in-house at ICAR-IVRI, Izatnagar.

The 96 well microtitre plates were coated with 50 µl of the working stock, diluted in coating buffer (Carbonate bicarbonate buffer, pH 9.6). The plate was incubated overnight at 4°C. Next day, it was washed with wash buffer (PBST, pH 7.2-7.4) thrice and blocked with 100 µl 5% skimmed milk powder in PBST. The plate was again incubated at 37°C for 1 hour. After washing the plate, 50 µl of working dilutions of three different standard control (strong positive, weak positive and negative sera) were dispensed to each well in four wells of the control panel. The test sera samples diluted (1:250) in PBST were added in duplicate well and incubated for 1 hr at 37°C. After washing thrice, 50 µl of 1:7000 diluted HRPO conjugated anti-bovine IgG in distilled water was dispensed in all the wells. Plates were again incubated at 37°C for one hour. Finally, after washing the plates thrice, 50 µl of the substrate (5 mg OPD tablet, Sigma) dissolved in 10 ml of distilled water containing 40 µl of 3% H_2O_2 was added to each well. The plates were incubated in dark for about 15 minutes at 37°C with intermittent observation of colour development. At the end of incubation, 50 µl of stop solution (1M H2SO4) was added to each well. The OD was measured in the ELISA plate reader (Microplate manager, BIO-RAD, USA) at 492 nm wavelength.

The per cent positivity (PP) value was calculated using the following formula.

Per cent positivity (PP) =

$$\frac{\text{OD of the test sample}}{\text{OD of strong positive (C++) serum}} \times 100$$

Samples having PP≥60 were considered positive, 50-60 were considered doubtful whereas below 50 were considered negative.

Statistical analysis

Chi-square and p-value were calculated to identify the significant difference ($p \leq 0.05$) in the prevalence of brucellosis among the various groups. The logistic regression (Binary) was performed to detect risk association of various factors (host and environment) on prevailing antibody. The risk analysis was done by taking *Brucella* positive and negative as dependant variables and the rest as covariates. Statistical analysis was done using SAS 9.3 software.

RESULTS AND DISCUSSION

Comparative spatial distribution of *Brucella* antibody among cattle of different rearing systems in different districts

Out of 920 field sera samples processed, the prevalence of brucellosis was recorded to be 21.3% (196/920) by i-ELISA and 14.24% (131/920) by RBPT. The diagnostic accuracy, sensitivity and specificity of indirect ELISA were recorded to be high (data not shown) against RBPT for screening of brucellosis.

Based on i-ELISA, a significant (χ^2=91.63, $p \leq 0.05$) variation in prevalence of brucellosis was recorded in all the five districts, with highest (44.75%, 81/181) in Balod district and lowest (4.84%, 9/186) in Rajnandgaon. A similar trend of significant variation (χ^2 =77.539, $p \leq 0.05$) was also recorded, based on "RBPT" (Fig 1). Prevalence of 33.85%, 32.61% and 30.90% brucellosis was recorded through ELISA, RBPT and STAT, respectively, among cattle in Pantnagar, India (Kushwaha *et al*. 2016). Prevalence of 21.36% brucellosis was recorded by Islam *et al*. (2013) in Punjab among cattle (28.17%) and buffaloes (15.12%). The variation in prevalence might be due to negligence of the disease or transboundary movement of the infected animal.

Among 17 organized farms (Fig 2a), prevalence of brucellosis was recorded to be highest in Dauji Dairy Farm

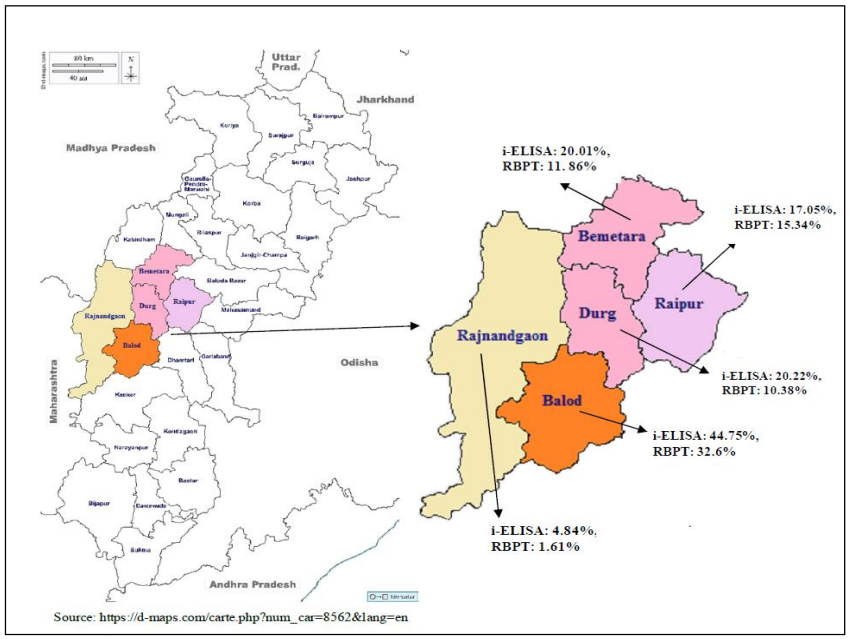

Fig 1: Spatial distribution of brucellosis in five districts of Chhattisgarh planes based on i-ELISA and RBPT.

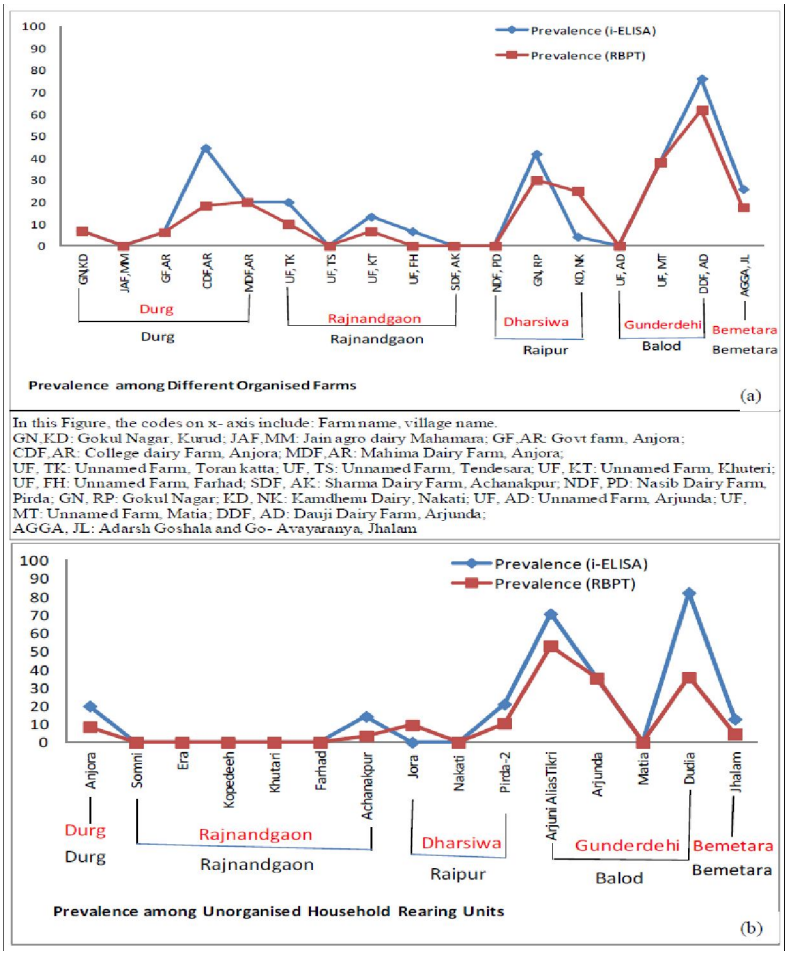

Fig 2: Prevalence of brucellosis among cattle of different organised farms (a) and unorganised household rearing units (b) in Chhattisgarh plains. The X-axis is showing the name of different organised farms and/village, blocks and districts.

Arjunda, Balod [i-ELISA: 76.19% (32/42); RBPT: 61.9% (26/42)] by both the diagnostic tests, while the lowest prevalence was recorded in Kamdhenu Dairy Nakati, Raipur (4.17%, 1/24) as per i-ELISA and Government Dairy Farm Anjora, Durg (6.25%, 1/16) as per RBPT. Similarly, prevalence among the unorganized household rearing units (Fig 2b) was also recorded by both the diagnostic tests. Based on i-ELISA, the prevalence was recorded to be highest in Dudia village, Balod (82.14%, 23/28) and lowest in Jhalam, Bemetara (12.79%, 11/86). As per RBP test, the prevalence was recorded to be highest in Arjuni Alias Tikri, Balod (52.94%, 9/17) and lowest in Achanakpur, Rajnandgaon (3.57%, 1/28). Number of farms and villages that were negative to brucellosis are depicted in Fig 2.

Prevalence of anti-*Brucella* antibody among cattle and risk association of different (host and environment) factors

The prevalence study was done with respect to different risk factors based on i-ELISA (Table 1) and risk association of different factors was analyzed through "Odds ratio" (OR) by binary logistic regression analysis (Table 2), where the last level of each independent variable was considered as reference category and risk association was analyzed by comparing positive sera to negative sera sample (healthy). Based on the analysis, sex (W=5.652, p=0.017) of the cattle significantly (p≤0.05) influenced the prevalence of anti-*Brucella* antibody, where female cattle had significantly (p≤0.05) higher prevalence (24.3%, 173/712) than that of male (Table 1). The OR indicated 2.044 times occurrence of *Brucella* antibody in female cattle in comparison to male (Table 2). This can be corroborated with the fact that female harbors infection causing reproductive problems, abortions and thus transmits the infection to healthy (Kabi *et al*. 2015; Ndazigaruye *et al*. 2018; Matope *et al.* 2011) through close interactions, following increasing herd size and stocking density (Megersa *et al*. 2011). The Wald statistics (W=0.153, p=0.997) revealed that the different breeds have an insignificant effect on the prevalence of brucellosis, while a significantly (p≤0.05) highest prevalence was recorded in Jersey crossbred cattle (53.57%, 45/84) and lowest in Gir. The variation in prevailing antibodies can be corroborated with the immune status of native cattle and susceptibility of crossbred (Patel *et al*. 2014), but the exotic cross breed cattle doesn't have a risk of prevalence than native cattle. The different age groups (W=0.335, p=0.846) have insignificant effect on the prevalence of brucellosis and also a non-significant (p>0.05) variation was recorded in prevalence of anti-*Brucella* antibody.

The Wald statistics (W=131.986, p≤0.001) revealed a significant difference (p≤0.05) in the prevalence of anti-*Brucella* antibody among the three seasons. A significantly (p≤0.05) highest seroprevalence was recorded during winter season (49.02%, 150/306) and lowest during summer (Table 1). Also, significantly (p≤0.05) lower OR was recorded during summer (0.063) and rainy season (0.062) than that of the winter (Table 2). There is no such theory exist explaining the correlation of brucellosis with seasonal variation, still the findings can be predicted due to the environmental stress (Tesfaye *et al*. 2011), restricting multiplication of bacteria/occurrence of more number of cases during sampling. The rearing system (W=2.659, p=0.103) has an insignificant risk associated with the prevalence, while, a significantly (p≤0.05) higher prevalence (24.69%, 118/478) was recorded among the organized farms in comparison to unorganized household rearing cattle units. The higher prevalence among the farms could be corroborated with conditions like close interaction within the herd, poor management practices, overcrowding, aborted materials, secretions and excretions from infected cattle (Kumar *et al*. 2016), but the cattle from organized farms doesn't have a risk of prevalence than that of cattle from unorganized household rearing units. The Wald statistics (W=69.817, p≤0.001) indicated that the prevalence

Table 1: Prevalence of brucellosis among cattle with respect to different factors as per i-ELISA.

Factor	Level	Prevalence % (P/N) by i-ELISA	$\chi^{2\text{-}p}$, where*p≤.05.NS p≥0.05
Nature of rearing	Organised farm cattle	24.69 (118/478)	6.787*
	Unorganised household rearing units	17.65 (78/442)	
Sex	Male	11.06 (23/208)	16.831*
	Female	24.3 (173/712)	
Age	<1 yr	24.11 (34/141)	0.813NS
	1-3 yr	20.5 (66/322)	
	>3 yr	21.01 (96/457)	
Breed	HF cross	13.64 (27/198)	68.08*
	Jersey cross	53.57 (45/84)	
	Sahiwal	19.87 (31/156)	
	Gir	8.7 (8/92)	
	Non-descriptive	21.79 (85/390)	
Season	Summer	7.19 (22/306)	210.101*
	Rainy	7.79 (24/308)	
	Winter	49.02 (150/306)	

Table 2: Risk analysis among different risk factors associated with prevalence of Brucellosis as per i-ELISA.

Variables in the equation (i-ELISA)

Risk factor and level	B	SE	W	DF	Sig	OR	95% CI for OR	
							Lower	Upper
Season			131.986	2	<0.001			
Summer	-2.757	0.289	90.771	1	<0.001	0.063	0.036	0.112
Rainy	-2.773	0.292	90.161	1	<0.001	0.062	0.035	0.111
Orgainsed	0.470	0.288	2.659	1	0.103	1.600	0.909	2.815
District			69.817	4	<0.001			
Durg	0.034	0.333	0.010	1	0.919	1.034	0.539	1.986
Rajnandgaon	-1.745	0.460	14.373	1	<0.001	0.175	0.071	0.430
Raipur	-0.213	0.399	0.286	1	0.593	0.808	0.370	1.765
Balod	1.599	0.347	21.205	1	<0.001	4.950	2.506	9.778
Breed			0.153	4	0.997			
HF cross	0.128	0.434	0.087	1	0.769	1.136	0.485	2.659
Jersey cross	0.081	0.468	0.030	1	0.863	1.084	0.433	2.712
Sahiwal	0.074	0.332	0.050	1	0.823	1.077	0.562	2.063
Gir	0.153	0.460	0.110	1	0.740	1.165	0.473	2.871
Age			0.335	2	0.846			
<1 year	0.169	0.294	0.332	1	0.565	1.185	0.666	2.107
1-3 year	0.054	0.232	0.054	1	0.815	1.056	0.669	1.665
Female	0.715	0.301	5.652	1	0.017	2.044	1.134	3.684
Constant	-1.022	0.384	7.081	1	0.008	0.360		

a. Variable(s) entered on step 1: season, sector, district, breed, age, sex.

of brucellosis varied significantly among different districts (Fig 1). A significantly highest seroprevalence was recorded in Balod district and lowest in Rajnandgaon. Also, significantly higher (4.950) and lower (0.175) OR were recorded in Balod and Rajandgaon respectively than that of Bemetara (reference district). This might be due to the introduction of more infected animals into the herd or close interaction with the infected through sharing pasture land (Manish *et al*. 2013).

The current study places on record endemicity of brucellosis covering the Chhattisgarh plains. Since all the samples during the study were collected from unvaccinated herds, the probability for the presence of vaccinal antibody is ruled out and the prevailing antibody can be corroborated with past exposure to brucellosis or ongoing infection. Moreover, antibody titres might not be detected in some animals possibly due to latent phase of infection, common in chronic brucellosis (Mai *et al*. 2012). Several factors (host and environment) pose risk to prevailing infection by superimposing the gravity of infection. This recalls for an urgent need of implementing strategic policy for control and prevention through proper vaccination to healthy, segregation and culling of infected/carriers animals (Verma *et al*. 2014). Thus, the present findings call for further detailed epidemiological investigation throughout the state with strategic control measures to reduce associated abortions and human health risks, in order to achieve the ''One Health''.

CONCLUSION

During the study, it was found that the plain zone of Chhattisgarh, India was endemic to Brucellosis, with a prevalence of 21.3% that was recorded through i-ELISA and 14.24% through RBPT. Prevalence of brucellosis has a significant ($p \leq 0.05$) odd's of association with various risk factors *viz*., sex (female), season (winter) and district (Balod). The associated factors are overlooked and the infection remains as a setback to livestock owners of Chhattisgarh plains. This research addresses endemicity of brucellosis, its associated problems and further alarms the animal keepers to take preventive and control measures to reduce the economic loss due to this disease.

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Animal ethical permission

This study requires no CPCSEA permission, since only sera sample were collected randomly and no animal experimentation was conducted.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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