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Seroprevalence and risk factors associated with bovine brucellosis in western Uttar Pradesh, India

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ABSTRACT

The present study was conducted to estimate the prevalence of brucellosis in cattle and buffaloes of western Uttar Pradesh, India and possible risk factors associated with it. Blood samples collected from 1,019 animals (dairy cattle and buffaloes) of different age and sex from 17 districts of western Uttar Pradesh, India where no vaccination against brucellosis is practiced were subjected to indirect ELISA for detection of *Brucella* antibodies. The overall mean seropositivity was 12.37% with seropositivity in unorganized and organized farms 4.08% and 24.88%, respectively. The young calves were having significantly higher seropositivity (10.38%) and it further increased in sexually matured adults (12.71%) in comparison to older animals (9.17%). Overall seropositivity and risk factors logistic regression analyses showed that species and farming type (organized and unorganized) had significant effect and sex of animals and their age group had no significant effect on the positivity of brucellosis. In absence of any vaccination presence of circulating antibodies against *Brucella* in all age group of animals indicated the natural circulation of infection in the state. Study indicated an urgent need of policy for prevention and control of brucellosis in dairy animals.

Key words: Brucellosis, Buffalo, Cattle, Risk factors, Seroprevalence

Brucellosis, a zoonotic disease having worldwide prevalence (Kumar *et al.* 2009, Manish *et al.* 2013) with more than 500,000 new cases in humans annually worldwide, is re-emherging with significant veterinary and public health concern (Manish *et al*. 2013). Bovine brucellosis is caused by *Brucella abortus* and sometimes by *Brucella melitensis* leads to abortion during the last trimester of gestation, weak newborn calves, and infertility (OIE 2008). India has vast regional and social values leading to variation in animal husbandry practices, which are major cause of variation in seroprevalence in different states and regions (Kumar *et al.* 2009, Saha *et al.* 2010, PD-ADMAS 2011, Khurana *et al.* 2012). In India, the estimated loss due to brucellosis is ₹ 350 million annually (Tiwari *et al.* 2013).

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'Risk factors' refer to aspects of the system that influence the introduction of a disease, or disease persistence, in the population and their identification could allow for effective disease management. Seroprevalence studies are prerequisite of control programme, so there is a growing need for serological surveillance of brucellosis in dairy animals. Hence, the present study was aimed to investigate the per cent positivity of antibodies to *Brucella* infection in dairy animals and possible risk factors associated with the disease in western Uttar Pradesh, India.

MATERIALS AND METHODS

Study area: A cross-sectional study was conducted in 17 districts (Agra, Aligarh, Budaun, Baghpat, Bareilly, Bijnor, Bulandsahar, Etah, Firozabad, Ghaziabad, Hardoi, Hathras, Jhansi, Kasganj, Mathura, Meerut and Saharanpur) of western part of Uttar Pradesh, India from 2011 to 2013. The climate of the investigated area is humid subtropical with dry winter type and the vegetation is tropical dry deciduous forest characterized by the rainfall of 650-1,000 mm/year, relative humidity (20-50%), and average annual temperature (11.0ºC to 36.9ºC).

Serum samples: All the animals were handled as per the guidelines of Ethical Committee. In this study, blood samples from 1,019 animals (of various sex, age and place) including 613 from unorganized and 406 from organized

farms. About 5 ml of blood was collected, aseptically from the jugular vein of each animal and brought to laboratory on ice. The serum was collected by centrifugation at 3,000 rpm for 5 min and stored at –20°C till laboratory test was performed.

Serodetection of anti brucella antibodies: The collected sera were screened for the presence of antibodies against *Brucella* antigens using a commercially available indirect enzyme linked immunosorbent assay (I-ELISA) as per the manufacturer's instructions.

Statistical analysis: The positivity of antibodies to *Brucella* infection was estimated using the ratio of positive results to the total number of animals examined. The epidemiological information about the animals was stored in a computer database and a chi-square test (Snedecor and Cochran 1994) was used to assess the differences between age and place/districts, with $P<0.01$ as the minimum level for statistical significance. The association between individual animal level determinants and seropositivity of brucellosis (odd ratio) was investigated using a logistic regression model. Receiver operating characteristic (ROC) curve analysis was performed by using software SPSS to show sensitivity and specificity.

RESULTS AND DISCUSSION

The study included 1,019 sera samples collected from organized and unorganized farms of 17 districts of western Uttar Pradesh, India. *Brucella* antibodies were detected in 126 of the 1,019 sera samples tested by ELISA and the positivity was 12.37% (Table 1). The overall seropositivity (12.37%) of brucellosis in animals is similar to findings of previous studies conducted in states, viz. West Bengal, Karnataka, Jammu and Kashmir, Uttar Pradesh, Gujarat, Punjab (Kumar *et al.* 2009). The seropositivity of brucellosis was higher in organized herd (24.88%) than 4.08% of unorganized herd (Table 1). These findings are in concurrence with the previous report of brucellosis in Jodhpur, Rajasthan, India (Kachhawaha *et al.* 2005). Similarly, higher rate of seropositivity was reported in organized farms from the Punjab region of Pakistan with the seropositivity in the range of 14.70% - 18.53% in cattle and 15.38% - 35.40% in buffaloes in government and private farms, respectively, (Nasir *et al.* 2004). The disease supposed to be more prevalent in areas of intensive animal husbandry practices such as organized farms (PD-ADMAS 2011) and the reason behind higher rate of seropositivity seems to be exposure of contaminated material and horizontal transmission of disease in organized farms because of poor management practices and overcrowding (Manish *et al.* 2013). Moreover, close contact between animals, high animal density, poor hygienic practices like improper disposal of aborted fetuses and fetal membranes and vaginal secretions, which ultimately help in rapid spread of infection from one animal to another in organized farms, might be the key reasons of higher seropositivity (Manish *et al.* 2013). Thus, overcrowding and mismanagement in organized farms are major risk factors involved in the spread of brucellosis.

Proportion of seropositive cases in cattle and buffalo were found similar in unorganized farm whereas overall, there was unequal distribution. Male and female animals were statistically found equally affected in all three categories (organized, unorganized and overall) but show slightly higher seropositivity in male animals as compared to female animals. An increasing seropositivity trend with increasing age was observed (Table 1). The district wise seropositivity of cattle and buffaloes from unorganized farms revealed that out of 16 districts, only 10 districts had seropositive animals either cattle or buffalo or both. So, the districts, which did not have seropositive cases, are not

Table 1. Seroprevalence of brucellosis for different factors in dairy animals

Factors	Organized farms			$\chi2$	Unorganized farms			$\chi2$	Overall seropositivity			$\chi2$
	Total animal	Seropositivity			Total animal	Seropositivity			Total animal	Seropositivity		
		Number	%			Number	%			Number	%	
Species												
Cattle	406	101	24.88	-	209	6	2.87	NS	615	107	17.4	6.5*
Buffaloes	Nil	Nil	Nil		404	19	4.7		404	19	4.7	
Sex												
Male	14	4	28.57	NS	69	6	8.69	NS	83	10	12.05	NS
Female	392	97	24.75		544	19	3.49		936	116	12.39	
Age												
< 1 year	55	7	12.73	NS	51	4	7.84	NS	106	11	10.38	NS
1.1-3 years	91	24	26.37		145	6	4.14		236	30	12.71	
3.1-5 years	46	14	30.43		161	6	3.73		207	20	9.66	
5.1-7 years	179	49	27.37		182	6	3.30		361	55	15.24	
>7 years	35	7	20.00		74	3	4.05		109	10	9.17	
Overall	406	101	24.88		613	25	4.08		1019	126	12.37	15.2**

Results are given as percent seropositivity; χ^2, stands for chi square tests; *, significantly different from other group within the same row ($P<0.05$); **, significantly different from other group within the same row ($P<0.01$); NS, not significant.

included in fig 1. There was variation in district wise seropositivity. In cattle, higher seropositivity of brucellosis was recorded from districts Bareilly (22.22%) and Bijnor (12.50%) whereas, in case of buffaloes it was highest in Baghpat (16.67%) and Hardoi (16.67%) followed by Bijnor (14.29%) (Fig.1). Overall per cent seropositivity also vary from district to district and it was highest in Hardoi (16.67%). This variation in seropositivity of brucellosis observed in different districts was very much expected and also in concurrence of previous report in an earlier study from the neighboring state Haryana, India (Khurana *et al.* 2012) and was almost similar to the pattern of different districts within the state and in between the state of Nigeria, which reported the variation of 0.2% to 80.0% in seropositivity of cattle (Mai *et al.* 2012). The reasons for the differences in seropositivity of brucellosis among the districts of the state could not be fully explained based on the available data, but may be related to differences in animal management practices like animal markets, purchase of infected animals for replacement or upgrading, demographic factors, climatic conditions, rearing of different species together and wild life interaction (Manish *et al.* 2013). Moreover, seropositivity is also affected with the herd size and permanence of animals in the herd. Large herd size of animals may enhance the exposure potential either by common feeding and watering point or increased contact among animals increasing the transmission of *Brucella* organism. In statistical analysis effect of herd size might be due to the more number of animals tested from large herds; making the probability of detection from individual animal greater than the animal from smaller herds (Solorio-Rivera *et al.* 2007)

Risk factors logistic regression analyses showed that species and farming type had significant effect and sex of animals and their age group did not show significant effect on the prevalence of brucellosis (Table 2). As expected, animals of organized farm had the highest prevalence of brucellosis and it varies from 4.92 to 12.32 times than that of organized farm; likewise disease prevalence was significantly higher in cattle than buffalo. Prevalence may be seen 2.57 to 7.07 times higher in cattle than buffalo. The possible reason for higher seropositivity in buffalo in comparison to cattle might be the practices of natural service in buffaloes. The overall seropositivity of male (12.05%) and females (12.59%) are almost identical with no significant difference and that might be due to the sample size and coincide the earlier reports that there is no difference in seropositivity of animals due to sex (Bayemi *et al.* 2009).

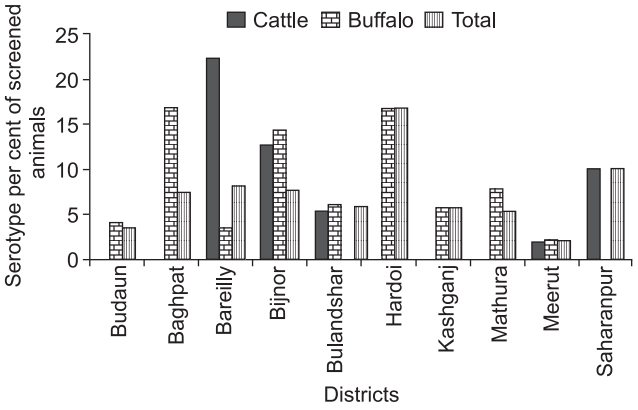

Fig. 1. Percentage seropositivity of animals for brucellosis in unorganized farm in different districts of Uttar Pradesh.

The age of the animals is always a crucial risk factor

Table 2. Logistic analysis of risk factor associated with Brucellosis

Factor	Group	Number examined	Found positive	Prevalence (%)	Odds ratio (95% CI)	P-Value
Species	Buffalo	404	19	4.7	1	0.001
	Cattle	615	107	17.4	4.268 (2.57–7.07)	
Sex	Male	83	10	12.05	1	0.92
	Female	936	116	12.39	1.03 (0.519–2.057)	
Farming type	Un-organized	613	25	4.08	1	0.001
	Organized	406	101	24.88	7.789 (4.921–12.327)	
Age group (Years)	<1	106	11	10.38	1	0.23
	1.1–3	236	30	12.7	1.25 (0.605–2.616)	
	3.1–5	207	20	9.6	0.924 (0.425–2.007)	
	5.1–7	361	55	15.2	1.55 (0.781–3.08)	
	>7	109	10	9.1	0.872 (0.354–2.149)	

associated with brucellosis. However, the seropositivity pattern varied in organized and unorganized farms (Table 1). These findings are similar to the studies conducted at Zimbabwe that seroprevalence of brucellosis decrease with increasing age (Mai *et al.* 2012). It is likely that in endemic areas, the risk of *Brucella* infection, and thus, sero-conversion, is greater in younger naive animals as compared to older ones. Moreover, some of which may not exhibit detectable antibody titers possibly due to latency, which is common in chronic brucellosis (Mai *et al*. 2012). Furthermore, the vertical transmission of infection might be the cause behind the higher rate of seropositivity in younger animals in endemic areas. The variation in the age at which sexual maturity is attained varies with breeds of cattle, and is likely to influence the number of seropositive animals in the age group and the relationship between age and positive reactors in different subpopulations. Although our observations about age and brucellosis seropositivity in organized farms (Table 1) coincide with other reports (Saha *et al.* 2010, Khurana *et al.* 2012). These findings corroborate those of previous findings and support the thought of higher positivity of brucellosis in older animals due to continuous exposure to infectious organism (Bandyopadhyay *et al.* 2009). Moreover, the preponderance of seropositive reactors in the above 2 years age groups may be related to the onset of sexual maturity, behavioral changes, estrous, which is associated with increased risk of infection with *Brucella* spp., especially following abortions (Manish *et al.* 2013).

The seroprevalence with multiple risk factors and different herds always have the possibility of false positive results affecting the final outcome of the study. However, the sensitivity of particular test in any study model reduces the chances of false positivity. For this a Receiver operating characteristic curve (or ROC curve) was plotted with true positive rate against the false positive rate for the different possible cut points of ELISA test (Fig. 2). Receiver operating characteristic (ROC) curve is the plot that depicts the trade-off between the sensitivity and (1-specificity) across a series of cut-off points when the diagnostic test is continuous or on ordinal scale. This is an effective method for assessing the performance of a diagnostic test. An ROC graph depicts relative tradeoffs between true positives and false positives. The area under the ROC curve was found 0.737 (0.694- 0.78 for CI-95%, P<0.001) that indicated variables like sample size, method of sample collection and target group (age, sex etc.) may lead to variation in test results thus ROC curve was plotted in the present study and can be considered to be "Fair" at separating seropositive from seronegative animals. Though, the present study is based on limited number of serum samples from 17 districts of Uttar Pradesh, India, which might not be a true representative of the target population, but the results are indicative of circulation of *Brucella* infection in the state as till date no vaccination is performed in this region against brucellosis. Moreover, due to continuous persistence of *Brucella abortus* infection (Kumar *et al.* 2009, Neha *et al.* 2014), inability to cull infected cattle due to religious issues and distress sale of animals following positive serological diagnosis raised an urgent need for the strict implementation of a control policy in cattle and compel government authorities to adopt a vaccination strategy to prevent and control brucellosis in cattle (Verma *et al.* 2014).

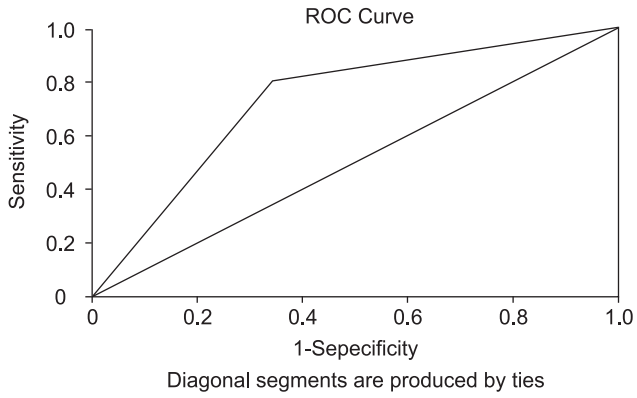

Fig. 2. Receiver operating characteristic (ROC) curve for ELISA in present study model.

The results of the present study provided serological evidence of widespread endemic presence of brucellosis in dairy cattle in western Uttar Pradesh, India. Various risk factors are associated to brucellosis and play crucial role in its spread. The presence of *brucella* antibodies in non vaccinated animal population is always suggestive of infection. The seropositivity in organized farms is at alarming level. Moreover, animal owners/farmers in this area are in close contact with these animals, and consumption of raw milk and improper handling of aborted materials is frequent. Thus brucellosis is not only the cause of reproductive and production losses but also may be the potential biohazard in this region. Thus, authors recommend further detailed epidemiological investigation to find out the link between animal and human brucellosis in the present study area and for formulation of strategic control measures in order to reduce associated reproductive wastage and the public health risks.

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