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SEROPREVALENCE OF BRUCELLOSIS AND ITS ASSOCIATED RISK FACTORS IN DAIRY ANIMALS FROM WESTERN PARTS OF UTTAR PRADESH, INDIA

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ABSTRACT

A cross-sectional study was performed in western parts of Uttar Pradesh, India to determine the prevalence of antibodies to *Brucella spp.* in dairy animals and study risk factors associated with disease. A total of 924 serum samples of dairy animals (860 cattle and 64 buffaloes) from ten districts located in the western part of Uttar Pradesh were collected with animal-level data such as species, sex, age, rearing practices and medical history. Serum samples were screened sequentially for the presence of *Brucella* antibodies using the Rose Bengal Plate test (RBPT) and indirect ELISA. The seroprevalence was found 8.45% and 20.45% in RBPT and iELISA, respectively. *Brucella* seroprevalence varied districtwise with highest rate in Baghpat (30.77%) and Agra (26.85%) districts. Depending upon the age seroprevalence also varied with higher rate in adults (=3 yrs) in comparison to young one (<3 yrs). However, there was no significant difference (P>0.05) in sex wise seroprevalence suggesting risk of brucellosis independent of sex in dairy animals. The organized farms revealed high *Brucella* seroprevalence in comparison to individually reared animals. Animals with history of abortion, retention of placenta and other reproductive disorders showed more seropositivity than controls. The study established the endemicity of brucellosis in dairy animals of western Uttar Pradesh. Considering the economic and public health aspect of the disease, the prevention and control measures are required to stop further spread of the disease.

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

India has the largest livestock population in the world with the vast majority of the cattle population of over 190 million reared under traditional animal husbandry practices. Although, there is high seroprevalence of brucellosis in animals but the infection does not receive much attention with respect to food borne zoonosis (1, 2, 3). Brucellosis has been reported with high endemicity in India, with national average of as high as 5% in cattle, 3% in buffalo, 7.9% in sheep and 2.2% in goats (4). Brucellosis is a globally recognized major zoonotic disease and prevalent in humans and domesticated animals (2, 3, 5). The variation in prevalence of brucellosis is mainly attributed to animal population dynamics, management and biological determinants like herd immunity, persistence of infection in calves etc (6). With the development of animal breeding and management practices, there is increase in movement of animals that is having the capacity to change the epidemiology of infectious diseases including brucellosis (7, 8, 9). There is dearth of information on its seroprevalence and associated risk factors in western parts of Uttar Pradesh. Therefore, this study was conducted to investigate the seroprevalence of brucellosis and factors associated with the occurrence of the disease in dairy animals from selected districts of western Uttar Pradesh, India.

MATERIALS AND METHODS

Study areas : A cross-sectional study was conducted was conducted in different districts viz., Agra, Amroha, Baghpat, Bulandsahar, Etawah, Firozabad, Ghaziabad, Hapur, Mainpuri and Mathura of western part of Uttar Pradesh, India from January, 2014 to April, 2015 (Figure 1). These districts were selected because of the high numbers of small holder dairy farmers with animal husbandry practices. The predominant cattle breeds are Zebu, Brahman and crossbreds of Holstein Friesian and Jersey, while the main buffalo breed is Murrah. The farmers used to keep various breeds of animals viz., local, crossbreds and some exotic breeds. The climate of this area is humid subtropical with dry winter. The meteorological parameters of the study area are - average annual temperature (11.0ºC to 36.9ºC); relative humidity (20-50%); annual rainfall (650-1000mm), and the vegetation are tropical dry deciduous forest. Agriculture with animal rearing is the predominant economic activity.

Study design and sampling of individual animals

This cross-sectional study was carried out using a convenient sampling procedure to select individual animal. The animals in the present study belonged to either organized farms or unorganized. In organized farms, animals were = 10, reared under semi-intensive system of management and animals were frequently in contact with other animals during feeding, watering and/or housing. In unorganized category, animals were individually reared by marginal or landless farmers relying on grazing in pastures and no or few supplementary feeding.

Table-1 : Brucellosis seroprevalence in dairy animals by species, sex, age group, rearing practice, health status and study area.

Parameters	Animal tested	Number of animals positive	
		By RBPT	By ELISA
Species			
Cattle	860	79 (9.17)	187 (21.69)
Buffalo	64	0	2 (3.13)
Total	924	79 (8.55)	189 (20.45)
Sex			
Male	21	0	2 (9.50)
Female	903	79 (8.75)	187 (20.71)
Age			
Young (<3 yrs)	27	0	3 (11.11)
Adult (=3 yrs)	898	79 (8.79)	186 (20.71)
Rearing Practice			
Individually reared	141	0	2 (1.41)
Organized herd	783	79 (10.09)	187 (23.88)
Health status			
Reproductive problems	89	18 (20.22)	44 (49.44)
Apparently healthy	835	61 (7.31)	145 (17.37)
Districts			
Agra	607	61 (10.05)	163 (26.85)
Amroha	07	0	0
Baghpat	39	13 (33.33)	12 (30.77)
Bulandsahar	12	0	0
Etawah	39	0	0
Firozabad	06	0	0
Ghaziabad	54	05 (9.26)	12 (22.22)
Hapur	46	0	0
Mainpuri	12	0	0
Mathura	102	0	2 (1.96)

Value in parentheses indicates prevalence in percentage

Epidemiological data and sample collection : Information on individual animal determinants viz., species, geographical location, sex, age, animal rearing practice and history of reproductive problems like abortion, repeat breeding and retention of placenta were also recorded separately on structured questionnaire. All the animals were handled as per the guidelines of Ethical Committee. In this study, about 3 ml of blood samples from 924 dairy animals (860 cattle and 64 buffaloes) were collected aseptically by juglar venipuncture and brought to laboratory on ice. The serum was collected by centrifugation at 3,000g for 15 min and and stored in cryovials at -20°C till further use.

Serological tests : Collected Sera samples were subjected to Rose Bengal Plate agglutination test (RBPT) (10) and Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) using commercially available kit (Svanova (Biotech-AB), Uppasala, Sweden) as per the manufacturer's protocol. Briefly, each of the 96 wells of flat bottom polystyrene antigen precoated ELISA plates (Nunc, Denmark) was brought to room temperature. Thereafter 90ìl of sample dilution buffer was added to each well used for serum sample and controls. Then 10ìl of serum samples were added in different wells along with 10ìl of positive control and 10ìl of negative control serum respectively to the selected wells coated with *Brucella abortus* antigen. After incubation at 37°C for 1 hr, plates were washed with washing solution (PBST) thrice. Then to the wells of ELISA plates, 100ìl of HRP conjugate was added and incubated at 37ºC for 1 hr. After incubation the plates were washed with washing solution (PBST) thrice and 100ìl of freshly prepared substrate solution was added to each well and incubated for 10 min at room temperature (18°C-25°C). The reaction was stopped by adding 50ìl of stop solution in each well and mixed thoroughly. Optical density (OD) of each well was measured at 450 nm with ELISA reader. Percentage positivity was calculated by the formula given belowa

$$\text{percentage positivity (PP)} \quad \frac{OD\ of\ sample}{OD\ of\ positive\ control} \quad 100$$

For individual sample, PP <40 was considered as negative, while PP >40 was considered as positive.

Statistical analysis : The epidemiological data were stored in a computer database using Microsoft excel spreadsheet (Microsoft Corporation) and statistical analysis was performed using SPSS version 14.0. The association between individual animal variables and seroprevalence of brucellosis was investigated using a logistic regression model.

RESULTS AND DISCUSSION

A total of 924 dairy animals (860 cattle and 64 buffaloes) from the ten districts of western Uttar Pradesh were tested for presence of antibodies to *Brucella spp.* using RBPT and ELISA tests. The number of individual animals that were positive for antibodies to *Brucella spp.* using RBPT and ELISA were 8.45% and 20.45%, respectively. The association of individual animal-level factors (species, sex, age groups, rearing practice, health status and districts) with brucellosis seroprevalence is shown in Table-1. The logistic analysis showed that species, age, rearing practice and health status were independently associated with *Brucella* seropositivity in animals (Table 2).

Species wise seroprevalence analysis revealed that distribution of antibodies against *Brucella* antigen was significantly (P<0.05) higher in cattle as compared to buffaloes. There was no difference (P>0.05) in seroprevalence between males and females animals. Brucellosis seroprevalence was observed to increase with increasing age of dairy animals. There were significantly higher (P<0.05) numbers of seropositive animals in adults (i.e. (=3 yrs age group) compared to those below 3 years. The seroprevalence of brucellosis was significantly higher (P<0.05) in organized herds in comparison to that of individually reared animals. The

Table-2 : Logistic regression model to predict the risk factors associated with brucellosis infection in dairy animals from different districts of Uttar Pradesh, India

Risk factor	Logistic regression				
	b	SE (b)	P value	OR	95% CI
Constant	-1.143	1.741	0.512	0.319	
Species	-1.987	0.726	0.006	0.137	0.033, 0.569
Age	0.151	0.057	0.008	1.163	1.040, 1.300
Rearing Practice	0.382	0.717	0.000	2.806	5.348, 8.914
Health status	0.787	0.892	0.004	2.078	4.478, 6.587

Results given with beta (b), standard errors (SE), and odds ratio (OR) with 95% confidence intervals (CI)

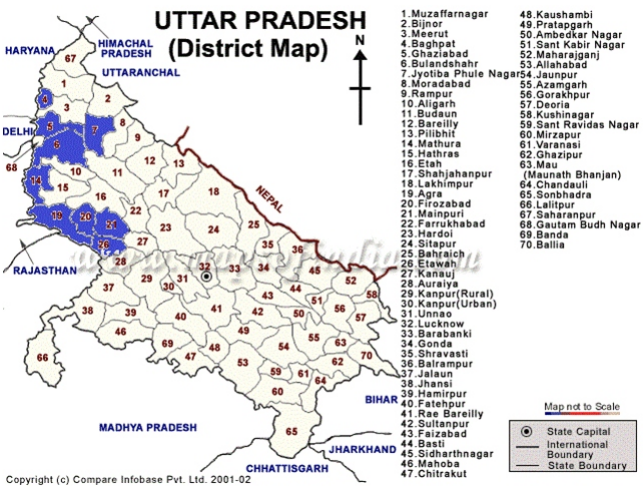

Figure-1 : Administrative map of Uttar Pradesh State showing the study area.

results also revealed that the seroprevalence of brucellosis was significantly higher (P<0.05) in animals having reproductive problems in comparison to that of apparently healthy animals or animals having some other problems.

In this study, brucellosis seroprevalence and the associated risk factors were investigated in dairy animals (cattle and buffalo) from different districts of western Uttar Pradesh, India. The study showed that brucellosis is present in the study area with seroprevalence of 8.45% and 20.45% using RBPT and ELISA, respectively. The seropositive reactions were likely to be caused by infection of Brucella spp. because the ELISA test used in the present study has high sensitivity and specificity, which minimises false-positive reactions caused by cross-reacting antibodies produced by certin organisms like Yersinia enterocolitica O:9, Escherichia coli O:157 and some Salmonella spp. (11). The observed brucellosis seroprevalence results agree with those of previous studies in different states of India (4, 12, 13, 14, 15). The factors that are attributable to this variation in prevalence rate of brucellosis are number of serum sample tested, number of farm from where the samples were obtained, whether samples belonged to government farms or private sector, samples originated from organized farm(s), serological assay(s) used for screening and samples collected from an endemic area or disease free area etc.

Species wise seroprevalence analysis revealed that distribution of antibodies against *Brucella* antigen were significantly (P<0.05) higher in cattle than buffaloes. Similarly, previous studies (15, 16, 17) also reported the higher prevalence of the brucellosis in cattle as compared to buffaloes. However, contrary to the findings, there are reports of higher seroprevalence of brucellosis in buffaloes than cattle (12), while no significant difference in seroprevalence of brucellosis in cattle and buffaloes (13). The possible reason for higher seroprevalence of brucellosis in cattle in comparison to that of buffaloes might be due to natural resistant in buffaloes to brucellosis (18). The natural resistance to *B. abortus* in buffaloes can be described by determining a correlation between the bovine brucellosis genotype and resistance to *B. abortus* infection (19). This variation in seropositivity can also be attributed to species susceptibility, as most of the cattle are crossbreds and exotic animals are more susceptible to stress conditions than buffaloes.

There was no significant difference (P>0.05) in seroprevalence between male and female animals, that suggested risk of brucellosis independent of sex of dairy animals as reported earlier (20). However, previous studies suggested higher seroprevalence of brucellosis in female animals (21), while contrary to this other (22, 23, 24, 25) reported the high prevalence of brucellosis in male animals.

Age is another vital determinant associated with the occurrence of bovine brucellosis. Sexually immature animals are supposed to be less susceptible to brucellosis than sexually mature animals (26). In the present study, seroprevalence of brucellosis was significantly higher (P<0.05) in animals older than 3 years, that is in agreement to the previous studies (6, 7, 9, 13, 27). The susceptibility of brucellosis increases as animal approaches the breeding age or sexual maturity (28, 29). This high seroprevalence in adult animals support the thought of higher positivity of brucellosis in older animals

due to continuous exposure to infectious organism (30, 31). Moreover, the preponderance of sero-positive reactors in the animals above 3 years age might be related to the onset of sexual maturity, behavioral changes, estrous, that is associated with increased risk of infection with *Brucella spp*., especially following abortions (30). Contrary to the present findings, higher seroprevalence was reported in animals aged 1-4 years of age (22, 25, 32) and this can be attributed to feeding of milk from infected dams (33), exposure to highly contaminated environment at unhygienic farms, and *in utero* transmission of disease from dam to fetus (26).

Association of bovine brucellosis infection with animal rearing pattern showed that the seroprevalence of bovine brucellosis was significantly higher ($p<0.05$) in animals of organized herd in comparison to individually reared animals. These findings are in accordance to previous study (34). This high prevalence of brucellosis in organized herd is attributable to horizontal transmission of infection as the infected animals contaminate the environment of dairy farms after abortion/delivery. Beside this, improper disposal of placenta and uterine discharges also contribute to unhygienic conditions help in transmission of disease from infected to uninfected animals (1, 15, 35). The presence of infected animal for a longer period of time on a farm increases the chances of more positive animals with time (26). Therefore, under such conditions prevalence of brucellosis gradually increases with time particularly in the absence of a control program. Therefore, overcrowding and poor management in organized farms are major risk factors involved in the spread of brucellosis.

Health status wise seroprevalence of bovine brucellosis revealed significantly higher ($p<0.05$) seroprevalence in animals having reproductive problems in comparison to apparently healthy animals as reported earlier (13, 27, 36). The primary source of *Brucella* organism in epidemiology of brucellosis in dairy animals is uterine fluid and placenta or aborted foetus expelled by infected cattle during abortion or parturition (37). Under optimum conditions, *Brucella* can survive for 66 days in moist soil and up to 185 days in cold soil (36, 37). Although not all abortions are due to brucellosis, but abortion is a major clinical sign of the disease in cattle and therefore should be suspected whenever observed (38).

District wise seroprevalence of brucellosis in dairy animals revealed wide prevalence of brucellosis in different districts. However, it could not be detected in six districts and that might be due to sampling error and small sample size. This variation in seroprevalence is in concurrence of previous studies conducted in Haryana, India (9) and Nigeria, which reported the variation of 0.2% to 80.0% in seropositivity of cattle (25). The reasons for this variation in district wise seroprevalence among the districts could not be fully explained based on the available data, but might be due 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, uncontrolled livestock movements, intermixing of animals and sharing of pasture lands as described previously (1, 30, 35).

From the study, it can be concluded that brucellosis is endemic in this part of Uttar Pradesh. However, district level differences in brucellosis seroprevalence could be attributed to management practices, livestock movement, mixing of animals etc. The seroprevalence did not differ between sexes of dairy animals, but increased with increasing age. Animals reared in organized herd and with a history of reproductive disorder were more likely to test seropositive for brucellosis. Taking the economic and public health aspect of the disease, the prevention and control measures like vaccination, regular screening and culling of positive reactors should be enforced for minimizing its prevalence and further reducing the risk to human beings.

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