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Seroprevalence of Brucellosis in Buffaloes by Indirect Enzyme Linked Immune-sorbent Assay in Punjab, India

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Abstract

The present cross sectional study was conducted to study the current status and epidemiology of brucellosis in organized Murrah buffaloes. A total of 628 animals were screened for antibodies against brucellosis using Enzyme Linked Immuno-Sorbet assay. An overall sero-prevalence of 15.12% (95/628) of brucellosis was observed in the present study. Statistically significant ($p < 0.01$) differences in susceptibility to brucellosis were observed with respect to sex (male 4%, 1/25 and female 15.58%, 94/603) and age. Significantly ($p < 0.05$) higher disease prevalence was observed in animals with a history of abortion than in those without such histories. A higher prevalence of brucellosis was recorded in the present study, which poses serious public and animal health threat. Therefore, a monitoring system needs to be in place to study the disease dynamics, so that the prevalence of the disease is brought down to an economically and biologically justifiable level.

Key words: Brucellosis, Sero-prevalence, Buffaloes

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Introduction

Brucellosis, a wide spread zoonosis is an important disease in humans, domestic and wild animals (Mustafa M, NicolettiP 1993, Tsolia *et al.,* 2002). The disease is caused by various species of the genus *Brucella,* which are classified based on preference of each species to its natural host (Quinn *et al.,* 1994). Bovine brucellosis is mainly caused by *Brucella abortus* and less frequently by *Brucella melitensis* (OIE, 2008). The infection is transmitted by direct or indirect contact with infected materials like aborted fetuses, placenta or infected animals and oral route serves as the main portal of entry of this agent (Crawford *et al.,* 1990). The disease in animals is characterized by abortion in females, retention of

placenta and infertility in both sexes (Omar *et al.*, 2002). The disease is worldwide in distribution. Serological evidence suggests that brucellosis is highly endemic in most parts of India (Mehra *et al.*, 2000, Sarumati *et al.*, 2003, Mittal *et al.*, 2005). The sero-prevalence of brucellosis in cattle ranged from 0.3% in Himachal Pradesh (Renukaradya *et al.*, 2002) to 56.2% in Assam (Chakraborty *et al.*, 2000). The sero-prevalence of brucellosis in buffaloes (*Bubalus bubalis*) has been reported from various regions of the country. Prahlad *et al*. (1999) reported a sero-prevalence of 11.14% in buffaloes from Delhi, from Madya Pradesh; Mehra *et al.* (2000) reported a sero-prevalence of 11.4% and from Punjab Sandhu *et al.* (2001) reported 9.33% sero-prevalence of brucellosis in buffaloes. The variation in prevalence of disease could be due to use of non-random sampling techniques (Dhand *et al.*, 2005). Most of these studies have used convenience or purposive sampling methods (Dhand *et al.*, 2005) and the information generated from these surveys cannot be extended to target population. The present study was, therefore, carried out to study the current status and epidemiology of brucellosis in buffaloes in Punjab.

Materials and Methods

Study Area

Punjab is the north-western state of India bordering with Pakistan on the west and situated between the 29.30° N to 32.32° N latitude and 73.55° E to 76.50° E longitude. Approximately 70% of human population lives in villages and agriculture is the main occupation. Murrah buffaloes and cross bred Holstein Frisian cattle are mostly reared in this region. The Murrah buffalo breed is also called the black gold of this region.

Design of the Study

The present cross sectional study was conducted on organized Murrah buffaloes from December 2010 to March 2012 in and around Ludhiana district of Punjab. Indirect Enzyme Linked Immuno-sorbent Assay (I-ELISA) and questionnaire were used to evaluate the sero-prevalence of brucellosis and host factors associated with the occurrence of brucellosis respectively. Host factors studied were age, sex and history of abortion. A sample size of 628 animals was selected using Win episcope -2 (95% confidence level, and ±5% desired level of accuracy).

Sample Collection

Blood samples were aseptically collected from the selected 628 buffaloes by jugular vein-puncture. About 5-10 ml of blood was collected in plain tubes without any anticoagulant. The blood samples were kept on ice immediately and until transported to laboratory. Serum was separated from clotted blood by centrifugation at 3000 rpm for 5 minutes and stored at - 20 $^{\circ}$C till further use.

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Serological Testing

Indirect-Enzyme Linked Immuno-Sorbent Assay (I-ELISA)

The test kit was purchased from 2-9, Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea (445-170). The kit contents were stored at 4-8 ºC until use. The test was performed as per the manufacturer's protocol. All the reagents of the kit and serum samples were brought to room temperature. Working dilutions of the reagents were prepared as per instructions of the manufacturer. Test samples were diluted 1:50 with sample diluent and 100µl of each diluted sample was transferred to a well in the micro-titre plate. Controls were run using 100µl of undiluted strong positive, weak positive and negative control into the designated wells. Mixing of plate contents was ensured by incubating the plate for 5 minutes in an orbital shaker followed by incubation of plate at 37 °C for one hour. After incubation the contents were discarded and the plate was washed five times with the wash buffer (PBS-Tween20). The plate was blot dried and 100µl of diluted enzyme conjugate was transferred to each well and incubated for 30 minutes. The contents of the plate were discarded followed by washing with wash buffer five times. After blot drying the plate, 100µl of ready to use substrate was transferred to each well followed by incubation for 15 minutes at room temperature. The reaction was stopped by addition of 100µl of stopping solution (1N sulphuric acid) to each well and absorbance of the wells was read at 450nm along with the reference wavelength of 620nm. The optical density (OD) values were used to calculate the percent positivity as shown in the equation. The test sera were categorised as positive or negative based upon the percent positivity value. Samples having percent positivity value 25 or above (%P ≥ 25) were categorised as positive and below 25 as negative (%P ≤ 25).

$$\%Positivity = \frac{OD\ of\ sample}{Average\ OD\ of\ standard\ strong\ positive\ control} \times 100$$

Statistical Analysis

Data from laboratory test and signalment of each animal were stored in excel spread sheet. The data were analysed using Chi Square (χ^2) test and Fisher's exact wherever needed.

Results and Discussions

Brucellosis is one of the major zoonotic problems throughout the world. Though some developed countries have been successful in eradicating this disease, but it still remains as one of the serious public health concerns in the developing world. The disease is endemic in most parts of India and there has seen a rapid increase in incidence and prevalence of the disease ever since it was first recognized in India in 1942 (Renukaradya *et al.*, 2002).

Sero-prevalence of brucellosis was estimated on the basis of results obtained from I-ELISA test assay. An overall sero-prevalence of 15.12% (95/628) of brucellosis was observed in the present study, which is

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higher than the prevalence reported previously (Dhand *et al.*, 2005), but lower than the prevalence reported by Varsada (2003). Present study was carried out in organized buffalo farms, which tend to have more animals per unit area. Large herd size enhances the exposure potential through increased contact between infected and non-infected animals, there by prompting transmission of the organism (Omer *et al.*, 2002). Moreover stocking densities have been found to be important determinant for *Brucella* transmission (Omer *et al.*, 2002).The disease is chronic in nature and infection may go unnoticed especially in case of heifers, males and young animals, which can lead to spatial clustering of cases. Generally male and female animals are equally susceptible to brucellosis. Statistically significant ($p < 0.01$) differences with respect to susceptibility to infection were observed among male (4%, 1/25) and female (15.58%, 94/603) animals in the present study. A higher sero-prevalence of brucellosis in female animals has been reported by various studies (Asfaw *et al.*, 1998, Muma *et al.*, 2007, Tolosa *et al.*, 2008, Bayemi *et al.*, 2009). The differences observed may be due to the fact that only 25 males were available for sampling in the study area, as most of the farmers opt for artificial insemination. Another aspect is that female animals are kept for longer in a particular herd and are stocked together compared to male animals which are individually housed, thereby increasing chances of exposure in females (Mekonnen *et al.*, 2010).

The results of the present study suggest that animals of more than 3 years of age were more likely to become sero-positive to brucellosis. The prevalence of brucellosis was higher in animals of age > 3 years (19.53%, 84/430) and least in animals of < 3 years (5.55%, 11/198) of age (Table 1).

Table 1: Sex and age wise sero-prevalence of brucellosis

S. No.		Tested	Positive	% Positive
Species	Buffaloes	628	95	15.12
Sex	Male	25	1	4
	Female	603	94	22.29
Age	< 3	198	11	5.55
	>3	430	84	19.53
Abortion History and Placental Retention				
		Total	Positive	%Positive
Aborted		21	16	76.91
Placental Retention		11	7	63.63
Trimester of Pregnancy at the Time of Abortion				
		Aborted	Positive	%Positive
2nd		5	1	20
3rd		16	15	93.75
Over all ser-prevalence, 95/628 = 15.12%				

The differences in the prevalence of the disease between these two age groups were statistically significant ($p < 0.01$), with animals in the age group of > 3 years being the most susceptible. Similar

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observations have been made by other workers (Sarumati *et al.*, 2003, Botha and Williamson 1989, Silva *et al.*, 2000, Amin *et al.*, 2005). Lower prevalence of brucellosis in young ones could be due to resistance of young animals to infection (Paul, 1980). Dhand *et al*. (2005) suggested that with passage of time animals are more likely to be exposed to the bacteria and contract the disease. However Kazi *et al.* (2005) is of the view that high prevalence of brucellosis among old animals might be related to maturity with advancing age, thereby the organism may have propagated to remain as latent infection or it may cause disease. Although susceptibility to brucellosis increases with age, it seems to be commonly associated with sexual maturity than age (Radostitis *et al.*, 2000).

With regard to history of abortion, only female animals greater than two years of age were included in analysis to avoid bias. Of the remaining animals, only 3.95% (21/531) were having history of abortion. Among the aborted animals 76.91% (16/21) were sero-positive. The sero-prevalence of brucellosis was significantly ($p < 0.05$) higher in animals with a history of abortion than in those without such histories (76.19%, 16/21). Out of the total 21 cases of abortion recorded in buffaloes, 16 cases of abortions occurred in the third trimester and 5 in the second trimester of gestation. Of these aborted animals 11 had history of retention of fetal membranes and seven (7/11) were sero-positive for brucellosis (Table 1). Statistically significant differences in sero-prevalence between animals with history of abortion and those without such histories were observed, these findings were in concurrence with that of Sandhu *et al.* (2001), who reported a higher sero-prevalence of brucellosis in animals with history of abortion. Most of these abortions were recorded in the last trimester of gestation and least in second trimester. The higher incidence of abortion in third trimester may be due to the fact that uterine environment becomes conducive for growth of *Brucellae* due to production of erythritol, which in turn causes damage to placenta and leads to abortion (Radostits *et al.*, 2000). Retention of placenta is a common sequel to abortion in brucellosis (Radostitis *et al.*, 2000). The prevalence of abortion and retention of placenta recorded in the present study was higher than reported previously (Tesfaye *et al.*, 2001).

Conclusion

A higher prevalence of brucellosis was recorded in the present study, which is a serious public and animal health threat. Therefore, a constant monitoring system needs to be in place to study the changes in the disease dynamics so that the control strategies can be manipulated to bring down the incidence and prevalence of the disease to a justifiable level.

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