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Seroprevalence of specific *Brucella* infection of cattle in Bangladesh Agricultural University Veterinary Clinics and its surrounding areas

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Abstract

A cross sectional survey was conducted to determine the seroprevalence of brucellosis in cattle in Bangladesh Agricultural University (BAU) Veterinary Clinics, in BAU Dairy Farm and Vabokhali from June 2008 to November 2008. A total of 200 serum samples were collected from BAU Veterinary Clinic, from BAU Dairy Farm and Vabokhali. Among the serum samples 143 sera samples were collected from BAU Veterinary Clinic, 42 serum samples from BAU Dairy Farm and 15 serum samples from Vabokhali. Sera were separated from blood samples and tested with specific *Brucella abortus* antigen (BAA) test and *B. melitensis* antigen (BMA) test. The overall seroprevalence of brucellosis in cattle was 5% in BAA and 0.5% in BMA. It was observed that, a significant higher prevalence of B. abortus was found in female than male. An insignificant higher prevalence of brucellosis was found in adult cattle (aged above 5 years), in cross breed cattle, in cattle with grazing, cattle breed by natural breeding, and in pregnant cows. Although insignificant but a higher prevalence of brucellosis was found in aged cattle than young cattle, cross bred cattle, pregnant cattle than non pregnant cattle, cattle with grazing. A higher prevalence of brucellosis was found in female cattle than male.

Key words : Cross sectional survey, Brucellosis, Cattle, Serological study

INTRODUCTION

Brucellosis is an important zoonotic bacterial disease (Matyas and Fujikura, 1984; WHO, 1986) caused by different species of the genus *Brucella*, that are pathogenic for a wide variety of animals and human beings (Mathur, 1971). According to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization of Animal Health (OIE), brucellosis is considered the most widespread zoonosis worldwide (Mustafa and Nicoletti, 1995).

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Brucellosis has been an emerging disease since the discovery of *B. melitensis* as the cause of Malta fever in the spleen of a fatal human case on the island of Malta in 1886, and isolated by David Bruce one year later in 1887, and *B. abortus* was isolated from the aborted cattle by Bernard Baisolate in 1897 (Nielsen and Duncan, 1990; Hatt-Jones, 2000). The first description of an outbreak of undulant fever caused by *B. abortus* involved college students who drank raw cows milk in the dormitory (Hugh-Jones , 2000).

Before 1945, India and Bangladesh were the same country and Bangladesh was belonged to Pakistan as East Pakistan till 1971. So historically, in this Indian

subcontinent, the credit of first investigation of contagious abortion in livestock associated with brucellosis, goes to the Imperial Veterinary Research Institute (now Indian Imperial Veterinary Research Institute), Muketswar, in northern India (Anonymyous, 1918). In Bangladesh, brucellosis was first identified in cattle in 1967 by Mia and Islam (1967), in buffalo in 1997 by Rahman et al (1997), and human brucellosis was first reported in 1983 by Rahman et al (1983).

The importance of brucellosis is not known precisely, but it can have a considerable impact on human and animal health, as well as on socioeconomic impacts, especially in which rural income relies largely on livestock breeding and dairy products (Islam et al, 1983). Human brucellosis is caused by exposure to livestock and livestock products. Infections can results from direct contact with infected animals and can be transmitted to consumers through raw milk and milk products. Most cases occur in people employed in meat processing industry while sources include the domestic cattle, pig, sheep, goat and unpasteurized dairy products (Radostits, 2000). In animals, the brucellosis mainly affects reproduction and fertility, reduces the survival of newborns, and reduce milk yield. Mortality of adult animals is insignificant (Sewell and Brocklesby, 1990).

Prevalence of brucellosis has been reported in cattle from different parts of the world. Rahman et al (1983) reported higher prevalence of brucellosis in cows of better managed farms and estimated of human brucellosis as 12.8% in herders and agricultural workers and 21.6% in goat farmers. Rahman et al (2006) reported the seroprevalence of brucellosis in cattle as 2.4~18.4% while the herd-level seroprevalence in cattle as 62.5% in Bangladesh. Azimun (2007) reported the seroprevalence of brucellosis as 4.5% in cattle and 6% in human.

In previous studies, reports of brucellosis in Bangladesh both in animal and human were made using only *B. abortus* antigen but there were no report of *B. abortus* and *B. melitensis* specific prevalence in cattle in Bangladesh. Therefore, the present study was carried out to study the prevalence of *B. abortus* and *B. melitensis* infection in cattle attending Bangladesh Agricultural University Veterinary Clinic and its surrounding areas.

MATERIALS AND METHODS

The study was conducted for a period of 6 months from June 2008 to November 2008 in the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Serum samples

A total of 200 serum samples were collected from BAU Veterinary Clinics and its surrounding areas. Among cattle sera samples, 143 samples were collected from BAU Veterinary Clinics, 42 samples were collected from BAU Dairy Farm and 15 samples were collected from Vabokolli. The study recorded some clinical, epidemiological and reproductive information. The questionnaire based data on age, gender, breed, area, client's complaint, pregnancy status, grazing pattern, types of housing and breeding, number of animals in herds, disease history, reproductive problems such as abnormal uterine discharge, abortion or previous abortion, repeat breeding in cows and reproductive diseases in bulls were recorded.

At first the animal was controlled by the owner and the attendant and then the site of blood collection at jugular furrow was soaked with tincture of iodine. About 4~7ml of blood was collected from jugular vein of each cattle with the help of sterile disposable syringe and needle and the blood was poured in a sterile vacutainer test tube and was kept undisturbed wan a tray for at least 30min. at room temperature in a slightly inclined position to facilitate clotting and separation of serum. After this period, the clotted blood samples with sera are transferred to refrigerator at 4°C and kept overnight. Then the blood samples with sera were centrifuged at 3,000rpm for 15min. After centrifugation a clear sera were found. Later on, the sera were pasred into the separate vial from each labeled vacutainer tube and the vial was marked with same number by permanent marker. The vial was stored in ice chamber at −20°C for use.

Serological study

B. abortus antigen and *B. melitensis* antigen test were used for the diagnosis of brucellosis.

B. abortus and *B. melitensis* antigen test

The test was performed according to the procedure as described by OIE (2004). The test serum samples and *B. abortus* and *B. melitensis* antigen (William James House Cowley Rd. Cambridt CB4 OWX U.K. www.atlas-site.co.uk.prepared by Atlas Msiscal) were kept 1 hour in room temperature before beginning of the test. Twenty microliters of each serum to be tested was placed on a glass plate circled approximately 2cm in diameter. Then the vial of antigens were shaked gently and one drop (50µl) of both antigen were put beside each of the sera. The antigens and the serum were mixed on the plate with a stirrer and spread over the entire area enclosed by the circle. Then the plate was placed on a mechanical rotator as 80~100rpm for 1 minute and the reading was taken immediately. The result was considered positive when there was any degree of agglutination noticeable and absense of agglutination considered as negative.

Data Processing and statistical analysis

The questionnaire based data were entered in Microsoft Excel 2003 and transferred to STATA® version 8.0/ intercooled (Stata Corporation, Texas, USA, 2003) for statistical analysis. Multiple logistic regressions were used to identify risk factors of brucellosis using software STATA®.

RESULTS

A total of 200 serum samples were collected to study seroprevalence of brucellosis in cattle. An overview of the data were presented in Table 1. The majority of cattle serum samples were collected from age group 0-5 years (122 out of 200) from BAU Veterinary Clinics (143 out of 200), from cross breed cattle (111 out of 200), from female (170 out 200), from cattle with parasitic infection (73 out of 200), from cattle with grazing (118 out of 200), from pregnant cattle (57 out of 200), from non pregnant cattle (143 out of 200), from cattle bred by natural breeding (47 out of 200), from cattle bred by artificial breeding (102 out of 200), from cattle accommodated with kaccha floor (130 out of 200), from cattle accommodated with brick floor (22 out of 200) from cattle accommodated with cemented floor (48 out of 200).

The overall seroprevalence of brucellosis in cattle based on age, gender, area, breed, clients complaint, grazing and housing type, pregnancy and breeding strategy was found to be 5.0% (10/200) and 0.5% (2/200) by *B. abortus* antigen (BAA) and *B. melitensis* antigen (BMA) test, respectively(Table 2).

The area-wise prevalence of brucellosis in cattle has been shown in Table 3. The higher prevalence of *B. abortus* was found to be in BAU Vet. Clinics (5.59%) than BAU Dairy Farm (4.76%) and Vabokhali (0.0%).

The higher prevalence of *B. melitensis* was found to

Table 1. Description of variables and their distribution for brucellosis data in cattle

Variables	Category level	No. of observation
Age (Years)	0-5 years	122
	Above 5 years	78
Area of sample collection	BAU Veterinary Clinics	143
	BAU Dairy Farm	42
	Vabokhali	15
Breed	Indigenous	89
	Cross	111
Gender	Male	30
	Female	170
Client's complain during sample collection	Anestrous	17
	Failure to concept	24
	For AI	13
	Mucorrhoea	9
	Retained placenta	14
	Parasitic infection	73
	Apparently healthy	50
Grazing	Yes	82
	No	118
Breeding	Natural	47
	Artificial	102
Housing type	Kaccha floor	130
	Brick floor	22
	Cemented floor	48
Pregnancy	Yes	57
	No	113

be in BAU Dairy Farm (2.38%) than BAU Vet. Clinics (0.70%) and Vabokhali (0.0%).

The age-wise distribution of brucellosis has been presented in Table 4. Cattle aged more than 5 years of age had higher prevalence (7.69%) and (2.56%) than the aged below 5 years (3.28%) and (0%).

The gender-wise prevalence of brucellosis has been shown in Table 5. A highly significant higher prevalence of *B. abortus* was found in female ($P<0.00$) (5.29%) than male. The higher prevalence of *B. melitensis* was found in female (1.18%) than male (0.0%).

The distribution of brucellosis in cattle according to the client's complaint has been has been shown in Table 6. The highest prevalence was found in cattle in cattle came for failure to concept (8.33%) and (4.17%) than other groups.

The breed-wise distribution of brucellosis was shown in Table 7. The higher prevalence of brucellosis was found in cross breed cattle (6.31%) and (1.80%) than indigenous cattle (3.37%) and (0%).

Table 2. Overall positive seroprevalence of brucellosis in cattle

Serum samples	Positive no. (%) by	
	BAA	BMA
200	10 (5)	2 (0.5)

Table 3. Area wise distribution of brucellosis in cattle

Area	No.*	BAA**		BMA***	
		Positive no. (%)	Total no. (%)	No. (%)	Total no. (%)
BAU Vet. Clinics	143	8 (5.59)		1 (0.70)	
BAU Dairy Farm	42	2 (4.76)	200 (5.0)	1 (2.38)	200 (0.5)
Vabokhali	15	0 (0.0)		0 (0.0)	

*Sera samples collected and tested, **Sera tested by BAA, ***Sera positive by BMA

Table 4. Age wise distribution of brucellosis in cattle

Age	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
0-5 years	122	4 (3.28)	200 (5)	0 (0)	200 (0.5)
Above 5 years	78	6 (7.69)		2 (2.56)	

Table 5. Gender-wise distribution of brucellosis in cattle

Sex	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
Male	30	1 (3.33)	200 (5.0)	0 (0.0)	200 (0.5)
Female	170	9 (5.29)		2 (1.18)	

Table 6. Distribution of brucellosis in cattle with client's complaint

Client's complaint	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
Failure to concept	24	2 (8.33)	200 (5.0)	1 (4.17)	200 (0.5)
Mucorrhoea	9	0 (0.00)		0 (0.00)	
Anestrous	17	1 (5.88)		0 (0.00)	
For AI	13	1 (7.69)		0 (0.00)	
Parasitic infection	73	3 (4.10)		0 (0.00)	
Retained placenta	14	1 (7.14)		0 (0.00)	
Apparently healthy	50	2 (4.00)		1 (2.00)	

Table 7. Breed wise distribution of brucellosis in cattle

Breed types	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
Indigenous	89	3 (3.37)	200 (5.0)	0 (0.0)	200 (0.5)
Cross	111	7 (6.31)		2 (1.80)	

Table 8. Distribution of brucellosis in cattle with grazing pattern

Grazing types	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
Yes	82	5 (6.10)	200 (5.0)	1 (1.22)	200 (0.5)
No	118	5 (4.24)		1 (0.85)	

Table 9. Distribution of brucellosis in cattle in pregnancy and non-pregnancy

Criteria of animals	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
Non-pregnancy	113	4 (3.54)	200 (5.0)	1 (0.88)	200 (0.5)
Pregnancy	57	5 (8.77)		1 (1.75)	

Table 10. Distribution of brucellosis in cattle with types of housing

Housing types	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
Kaccha 130	130	6 (4.66)	200 (5.0)	1 (0.77)	200 (0.5)
Cemented	48	3 (6.25)		1 (2.08)	
Brick	22	1 (4.55)		0 (0.00)	

Table 11. Distribution of brucellosis in cattle with types of breeding

Breeding types	No. of sera collected and tested	Sera positive by BAA		Sera positive by BMA	
		No. (%)	Total no. (%)	No. (%)	Total no. (%)
Natural	47	4 (8.51)	200 (5.0)	1 (2.13)	200 (0.5)
Artificial	102	6 (5.88)		1 (0.98)	

The distribution of brucellosis on the basis of grazing has been shown in Table 8. Most positive cases were found in cattle with grazing (6.10%) and (1.22%) than cattle without grazing (4.24%) and (0.85%).

The distribution of brucellosis with pregnancy has been shown in Table 9. A higher prevalence was found in pregnant cattle (8.77%) and (1.75%) than non pregnant cattle (3.54%) and (0.88%).

The distribution of brucellosis with housing type in cattle has been has been shown in Table 10. The highest prevalence was found to be in cemented floor (6.25%) and (2.08%) than kaccha (4.66%) and (0.77%) and Brick floor (4.55%) and (0%).

The distribution of brucellosis according to types of breeding has been shown in Table 11. The more positive cases were found in cattle bred by natural breeding (8.51%) and (2.13%) than by the artificial one (5.88%) and (0.98%).

As we got only two positive cases of *B. melitensis*, so it was not possible to analyze association between outcome variable and independent variable.

DISCUSSION

A total of 200 sera samples were collected to study the seroprevalence of brucellosis in cattle. The overall seroprevalence of brucellosis in cattle was 5% (10/200) and 0.5% (2/200) which is higher than the overall seroprevalence of brucellosis; 2% (250) reported by Amin et al (2004). 3.14% reported by Darwish and Benkirane (2001) and 3.8% Mruanlini and Ramasastry (1999). It is also agreement with Rahman et al (2006) who reported animal level seroprevalence of brucellosis in cattle is 2.4~18.4% while the herd level seropre-valence in cattle is 62.5%.

Cattle aged more than 5 years age had insignificantly higher prevalence of 7.69% and 2.56% than that aged

below 5 years. This findings correlate with the observation of Sarumathi et al, 2003; Kubuafor et al, 2000; Rajesh et al, 2003; Amin et al, 2004). So, it is considered that the higher prevalence of brucellosis among older cattle might be due to m% anity with the advanced age. However, the older animals supposed to be more infected, because of more contact of the infectious agents and sometimes from malnutrition during pregnancy.

The prevalence of brucellosis in cattle was found to be higher in females (5.29% and 1.18%) than male (3.33% and 0%). This finding was similar to the findings recorded by Sharma et al (2003).

The prevalence of brucellosis was found to be lower in indigenous breed (3.37% and 0%) by BAA and BMA than cross breed (6.31% and 1.80%). This may due to genetic factors that made indigenous breed resistant to the infection.

The prevalence of brucellosis was found to be higher in pregnant cows (8.77% and 1.75%) than non pregnant cows (2.79% and 0.88%). Similar results were reported by Amin et al (2005), they recorded 5.9% in pregnant cows than in non-pregnant cows (4.7%).

The prevalence of brucellosis was higher in cattle with grazing (6.10% and 1.22%) than non grazing (4.24 % and 0.85%). It was reported by Silva et al (2000). The author stated that this may be due to the unrestricted contact between animals.

The prevalence of brucellosis in cattle by natural breeding (8.51% and 2.13%) was found to be higher than cattle breed by AI (5.88% and 0.98%). It was reported by Sarumathi et al (2003). The study stated that the higher prevalence of brucellosis in cattle bred by natural breeding (5.72%) may be due to presence of infectious bulls used for natural breeding.

The presence study revealed that the 4.66%, 4.55%, and 6.25% in cattle accommodated with Kaccha, Brick and Cemented floor, respectively, were seropositive to brucellosis. This results imply that infection of brucellosis may vary with types of animal housing which is similar to the study of Ghani et al (1998).

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