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Seroprevalence of brucellosis in buffaloes in Bagerhat and Mymensingh district, Bangladesh

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ABSTRACT

An experimental survey was conducted to determine the seroprevalence of brucellosis in buffaloes in Bagerhat and Mymensingh regions of Bangladesh. A total of 70 sera samples were collected from the study of which 24 were collected from Bagerhat and 46 were collected from Mymensingh district. Questionnaire based data on risk factors were collected for buffaloes. The samples were screened by Rose Bengal Test (RBT). Positive, doubtful and negative samples were further confirmed with indirect Enzyme Linked Immunosorbent Assay (i-ELISA). The overall serological prevalence of brucellosis was 4.28%. It was observed that, a higher prevalence of brucellosis was found in female than male; aged animal than young animal with reproductive disorder than animal without reproductive disorder and a history of previous abortion was associated with the higher prevalence of brucellosis than that of other reproductive diseases. The result showed that female animal has more possibilities of infection to brucellosis. Among the 1.3 million of buffaloes in Bangladesh, a considerable number of buffaloes have reproductive dificulties and *Brucella* infection is one of them. Since brucellosis is a zoonotic disease and a number of human being are at risk, the detailed epidemiological studies along with prevention and control strategies are necessary.

Key words: seroprevalence, brucellosis, buffaloes, Bangladesh

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INTRODUCTION

Brucellosis is an important disease caused by gram negative bacteria *Brucella* that are pathogenic for a wide variety of animals and human. The disease is also called 'Malta fever', 'Mediterranean fever or undulant fever'. Almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection (Moore and Chnurrenberger, 1981; OIE, 2000). The species of *Brucella* and their major hosts are *B. abortus* (cattle and buffaloes), *B. melitensis* (goats), *B. suis* (swine) and *B. ovis* (sheep). Brucellosis is considered to be an occupational disease that mainly affects slaughter house workers, butchers, and veterinarians. Transmission typically occurs through contact with infected animals or materials with skin abrasions. However, the importance of brucellosis was primarily due to its public health significance and economic loss to the animal industry (WHO, 1971).

In animals, *Brucella* mainly affect reproduction and fertility, characterized by abortion, retained placenta, orchitis and subsequent high rate of infertility, reduces milk yield and the survival of newborns. Mortality of adult animal is insignificant (Sewell *et al.*, 1990). Brucellosis in buffalo is caused by *B. abortus,* causes chronic inflammatory lesions in the reproductive organs of both sexes, with occasional localization and lesions in other tissues. In human beings, the classical symptoms of the disease are weakness, joint & muscle pain, headache, undulant or intermittent fever, hepatomegaly, spleenomegaly, night sweats & chills, marked asthenia & anorexia (Hugh- Jones, 2000).

The livestock sector in Bangladesh plays an important role in the national economy. The buffalo population in Bangladesh is about 1.3 million (Banglapedia, national encyclopedia of Bangladesh). Buffalo production in Bangladesh is declining, as evidenced by reducing buffalo population and lack of enough research work on diseases of buffaloes (Huque, 2010). There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which is thought to cause by *Brucella* and play an important constraint for the development of livestock in Bangladesh (Rahman *et al*., 2006). However, there is little or no report of brucellosis alongside its association with reproductive diseases in buffaloes in Bangladesh. Rose Bengal Test (RBT), and Enzyme Linked Immunosorbent Assay (i-ELISA) were used for the detection of *Brucella* infection in buffaloes in this study. The association between *Brucell*a infections and other reproductive and no reproductive diseases were also investigated.

MATERIALS AND METHODS

Collection of sample and data

A total of 70 buffaloes were randomly selected for blood collection from buffaloes of two different areas (Bagerhat and Mymensingh) of Bangladesh. In Bagerhat, the samples were mainly collected from buffalo breeding and development farm, Bagerhat sadar and its villages. In Mymensingh, samples were collected from dairy farms of Bangladesh Agricultural University, Mymensingh sadar, slaughter house and some villages of Mymensingh district. Buffaloes were selected randomly for this study.

The blood samples were collected randomly and aseptically from both sexes of buffalo populations. A total of 70 blood samples were collected from buffaloes in selective areas of Bagerhat and Mymensingh districts of Bangladesh. Among the samples, 24 (8 male and 16 female) were collected from Bagerhat and 46 (18 male and 28 female) were from Mymensingh region. During sampling, a questionnaire based data on age, sex, area, client's complaint, pregnancy status, number of animals in herds, disease history, reproductive problems such as abnormal uterine discharge, abortion, retain placenta, repeat breeding & other reproductive diseases in female buffaloes were recorded. The sera samples were collected from age group of 1.5-6 years of which 44 were female and the rest 26 were male (table 1).

About 5-7 ml of blood was aseptically collected from jugular vein of buffalo with the help of sterile disposable syringe and needle (10 ml) and was kept undisturbed on a tray or other suitable place for at least 1 hour at room temperature with a slightly inclined position to a facilitate clotting and separation of serum. After this period, the clotted blood samples with sera are transferred to refrigerator at 4°C and kept for overnight. Later on, the sera were poured into the separate test tubes and properly labeled. The sera were centrifuged at 300 rpm for 10 minutes; supernatant containing clean sera were transferred to the sterilized eppendorf tube and stored at -20^{o}C until used.

Serological study

The serological test for the diagnosis of brucellosis in buffalo was performed by Rose Bengal Test (RBT) as screening test and indirect Enzyme Linked Immunosorbent Assay (i-ELISA) as confirmatory test.

Rose Bengal Test (RBT)

The test was performed according to the procedure as described by OIE (2000). The test serum samples and *B. abortus* antigen (Atlas Medical, UK) were kept 1 hour at room temperature before starting of the test. Fifty (50) µl of each serum to be tested was placed on a glass plate circled approximately 2 cm in diameter. Then the vial of antigens was shacked gently and one (1) drop (50µl) of antigen was put beside each of the serum. The antigens and the serum were mixed in the plate with a stirrer and spread over the entire area enclosed by the circle. Then the plate was placed on a mechanical rotator at 80-100 rpm for 3-4 minutes and the reading was taken immediately. The result was considered as positive when any degree of agglutination noticed.

Indirect Enzyme Linked Immunosorbent Assay (i-ELISA)

The assay was performed according the protocol and suppliances provided by the ELISA kit manufacturer company (Svanova Biotech AB, Uppsala, Sweden). All reagents supplied by the manufacturer company were equilibrated to room temperature (18 to 25°C) before use. A 100 µl of sample dilution buffer was added to each well having serum samples and control serum. Four 4 µl of positive control serum (reagent A) and 4 µl of negative control serum (reagent B) were added, respectively to the selected wells coated with *B. abortus* antigen. Samples were run in duplicates. The plate was shaked thoroughly and sealed followed by incubated at 37°C for l hour. The plate was rinsed 3 times with PBS-Tween buffer. Then, 100 µl of HRP conjugate was added to each well and incubated at 37°C for 1 hour. Again, the plate was rinsed and 100 µl substrate solution was added to each well and incubated for 10 minutes at room temperature. The reaction was stopped by adding 50 µl of stop solution (10% H_2SO_4) to each well and mixed thoroughly. The stop solution was added in the same order as the substrate solution was added. The optical density (OD) of the controls and test samples were measured at 450 nm in a microplate photometer. The OD was measured within 15 minutes after the addition of stop solution to prevent fluctuation in OD values. The percent positivity value (PP) was calculated as follows:

$$\text{PP} = \frac{\text{Test sample or negative control (OD)}}{\text{Positive control (OD)}} \times 100$$

The assay was calibrated against the DIE ELISA standard sera and standardized against the EU directives 64/432/EEC. PP values in sera of ≥25, are considered to be positive, and below this value are considered to be negative.

Table 1
An overall information on collected sera samples from buffaloes

Variables	Category level	Number of observation
Area of sample collection	Bagerhat region	24
	Mymensingh region	46
Age	Below 4 years	48
	Above 4 years	22
Gender	Male	26
	Female	44
Pregnancy	Yes	8
	No	36
Breeding type	Artificial breeding	26
	Natural breeding	12
Grazing	Yes	28
	No	42
Reproductive complain	Anestrous	6
	Retain placenta	4
	Abortion	3
	Repeat breeding	7
	Mastitis	1
	Vaginal discharge	8
	Dystocia	2
	Balanoposthitis	1

Statistical analysis

The questionnaire-based data was processed by Microsoft Excel and MSTATC. The results were analysed for significance by using chi-squared analysis determined at 5 percent.

RESULTS AND DISCUSSION

The objectives of this study were to investigate the serological status of buffaloes in selected areas of Bagerhat and Mymensingh districts of Bangladesh. RBT and i-ELISA were used to detect brucellosis in buffaloes and determined the association of brucellosis with some common non reproductive and reproductive factors.

Overall seroprevalence of brucellosis in buffaloes

The overall seroprevalence of brucellosis in buffaloes was shown in table 2. The overall prevalence of brucellosis observed by RBT was 5.71% and by i-ELISA was 4.28% confirming the better sensitivity of brucellosis on RBT. The overall prevalence of brucellosis in Bagerhat was 4.17% and in Mymensingh 4.35%, showing that the prevalence in Mymensingh region was 1.04 times more than that of Bagerhat region. The overall prevalence of brucellosis in buffaloes in this study was higher than that of 1.8% reported by Isloor *et al*. (1998) and 4.18% reported by Mishra *et al* (2006) but lower than the seroprevalence found as 8.75% by Rao *et al*. (1999), 6.92% by Iftikhar *et al*. (2008), 6.67% by Vikrant *et al*. (2005) and 19.12% by Brahmabhatt *et al*. (2009). However the variation of the prevalence rate in different studies might be due to variation in regions, methodology for detection of pathogens and other factors.

Factors associated with the prevalence of brucellosis

Association with non reproductive disorders

Age related seroprevalance of brucellosis in buffaloes was shown in table 3. The results of this study showed that buffalo aged more than 4 years had higher prevalence than that of the age group below 4 years in both tests. There was no significant relationship between age and occurrence of brucellosis. However, the older animals supposed to be more infected, because of more contact with infectious agents and sometimes from malnutrition and during pregnancy.

The prevalence of brucellosis in buffaloes was found to be higher in female than male using both RBT and i-ELISA. The difference between the sex groups was not statistically significant. This finding appeared similar to the findings of Das *et al*. (2004). A higher prevalence was found in pregnant buffaloes than non pregnant buffaloes and it was 12.50% and 5.55% by RBT, whereas 12.50% and 3.33% by i-ELISA, respectively. The occurrence of brucellosis had no significant relationship with pregnancy status. The difference between the groups was not statistically significant. The finding correlates with the observation of Chauhan *et al. (*2000). More positive cases were found in buffaloes without grazing (4.76%-7.12 %) than that of buffaloes with grazing (3.57 %) and the association of grazing or no grazing buffaloes with the prevalece brucellosis was statistically non significant.

Table 2
Overall sero-prevalence of brucellosis in buffaloes based on RBT and i-ELISA

Areas	Total number of sera samples collected and tested	Total number and % of positive reactor by RBT	Total number and % positive reactor by i-ELISA	Overall sero-prevalence	Odds ratio
Bagerhat	24	1 (4.17%)	1 (4.17%)	1 (4.17%)	1
Mymensingh	46	3 (6.52%)	2 (4.35%)	2 (4.35%)	1.04
Total	70	4 (5.71%)	3 (4.28%)	3 (4.28%)	

Table 3
Association of non reproductive disorder with seroprevalence of brucellosis in buffaloes

Species	Age of buffaloes	Number of sera samples collected and tested	Number and % of sera positive by RBT	Number and % of positive reactor by i-ELISA
Age	Below 4 yrs	48	2 (4.17%)	1 (2.08%)
	Above 4 yrs	22	2 (9.09%)	2 (9.09%)
Sex	Male	26	1 (3.85)	1 (3.85)
	Female	44	3 (6.82)	2 (4.55)
Pregnancy	Pregnant	8	1 (12.5%)	1 (12.5%)
	Non pregnant	36	2 (5.55%)	1 (3.33%)
Grazing	Yes	28	1 (3.57)	1 (3.57)
	No	42	3 (7.12)	2 (4.76)
Breeding	Artificial breeding	26	1 (3.84%)	1 (3.84%)
	Natural breeding	12	2 (16.67%)	1 (8.33%)

Table 4
Association of reproductive disorder with seroprevalence of brucellosis in buffaloes

Types of reproductive disorders	Number of sera samples collected and tested	Number & % of sera positive by RBT	Number and % of positive reactor by I- ELISA
Anestrous	6	1 (16.67%)	1 (16.67%)
Retain placenta	4	1 (25.00%)	0 (0.00%)
Abortion	3	1 (33.33%)*	1 (33.33%)*
Mastitis	1	0 (0.00%)	0 (0.00%)
Repeat breeding	7	0 (0.00%)	0 (0.00%)
Vaginal discharge	8	0 (0.00%)	0 (0.00%)
Dysticia	2	0 (0.00%)	0 (0.00%)
Balanophosthitis	1	0 (0.00%)	0 (0.00%)
Total	26	3 (11.54%)	2 (7.69%)

* =significant at 5% level of probability ($p<0.05$)

In this study, the prevalece rate was found higher in buffaloes of natural breeding that of buffaloes breeded by artificial insemination. The prevalence was 16.67% by RBT and 8.33% by i-ELISA in natural breeding. Whereas, the prevalence was 3.84% by both RBT and i-ELISA in artificial insemination. The association among the breeding types and the prevalence of brucellosis were noted non significant.

Association with reproductive disorders

In buffaloes with history of anestrous, the prevalence of brucellosis was 16.67% by both

RBT and i-ELISA. The prevalence of brucellosis in buffaloes with history of retained placenta was 25.00% by RBT but 0.00% by i-ELISA. In buffaloes with history of previous abortion, the prevalence of brucellosis was 33.33% by RBT and 0.00% by i-ELISA. The data showed that the prevalence of brucellosis was significantly higher in aborted or previously aborted buffaloes than that of buffaloes having no record of abortion (table 4).

In conclusion the study confirm the presence of brucellosis in buffaloes of the selected areas. Several factors observed in this study have influences on the prevalecnce of brucellosis in buffaloes. The factors such as age, sex, breed, location, herd size and living condition influence the seroprevalence of brucellosis stated by Ghani et al., (1998). However, further studies on molecular epidemiology of brucellosis are needed in order to better understanding the transmission dynamics and asses the human health risk by animal brucellosis in Bangladesh.

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