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Seroprevalence of brucellosis in sheep in Mymensingh and Netrokona district of Bangladesh

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ABSTRACT

Brucellosis is an economically important disease worldwide. Bangladesh has been reported an endemic area for brucellosis. The occurrence of brucellosis in people and the distribution of the disease among the livestock to be spatially related. A survey of brucellosis was carried out in sheep of Netrakona and Mymensingh districts of Bangladesh. A total number 102 blood samples were collected from sheep of the selected areas. Information of sheep's age, sex, location, pregnancy status, abortion, reproductive disorder were collected and tested for *Brucella* specific antibody response by RBT (Rose Bengal Test) and iELISA (Indirect Enzyme Linked Immunosorbent Assay). The overall serological prevalence of brucellosis was recorded 9.80% in sheep. The prevalence of brucellosis in male sheep was higher than in female in Mymensingh and vice versa in Netrokon district. The overall prevalence of brucellosis was higher in sheep with abortion than sheep with no abortion. The higher rate of *Brucella abortus* antibody was recorded in sheep above 2 years of age.The study revealed that the risk of being infected by brucellosis of sheep in Netrakona is 1.32 times higher than that of a sheep in Mymensingh. The result suggests that female sheep has an increased chance of brucellosis than a male. The prevalence of brucellosis was higher in sheep with history of abortion, and pregnancy compared to sheep with history of anestrus and failure to conception. The results of the study provide a baseline data for further study of *Brucella ovis* infections in the area and a landmark for taking control measures of brucellosis.

Key words: brucellosis, seroprevalence, mymensingh, netrokona

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INTRODUCTION

Brucellosis is a zoonotic disease affecting many species of domestic animals and man. The disease is characterized by abortion and other disorders of reproductive system in affected induviduals. Brucellosis mainly affects reproduction and fertility, reduces the survival rate of newborns and reduce milk yield (Roth *et al.*, 2003; Franco *et al.*, 2007) Different species of *Brucella* (small non-motile coccobacilli shaped gram-negative bacteria belong to the genus *Brucella*) are responsible for the abortion in animal and women (Young, 1995). Brucellosis in sheep caused by *Brucella ovis*, one of the most virulent species of *Brucella*, is a widespread zoonosis, especially in Mediterranean and the middle-east regions where it also constitutes a hazard for humans (Jacques *et al.*, 1998). In humans, ovine/caprine brucellosis caused by *Brucella melitensis* is by far the most important clinically apparent disease (Amato Gauci, 1995).

Sheep are important part of global agricultural economy. In Bangladesh sheep are mainly utilized for meat purposes and also important good quality leather and source of income to farmers. The total number of sheep in Bangladesh is about 2.8 million (Bangladesh Economic Review-2008 and DLS-2009). In Bangladesh, approximately 80 per

cent of people live in villages, and rural income is largely dependent on livestock; the people are in close contact with livestock on a daily basis. There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which is thought to be brucellosis. This brucellosis in animals plays an important constraint for the development of livestock in Bangladesh. The importance of brucellosis in Bangladesh is not known precisely, but it may have a considerable impact on both human and animal health, as well as having socioeconomic effects.

Bangladesh has been reported as an endemic area for brucellosis because of a considerable number of human and animal populations are exposed to the infection each year (Rahman *et al.,* 1978). Definitive diagnosis of brucellosis can be accomplished only through the direct demonstration and identification of the causative agent(s) by culture and isolation procedures (Orduna *et al,* 2000). Accurate presumptive diagnosis can be achieved from serological techniques used in combination with clinical observations and case histories. Although classical serological techniques suffer from several drawbacks, poor performance and lack of standardization, RBT is used as a screening test of *Brucella* infection (MacMillan, 1990) and more sensitive than the CFT when testing culture positive animal (Blasco *et al.,* 1994) and ELISA techniques have the potential as epidemiological tool for investigating the infective status of flocks (Rahman, 2003). Clinical cases of animal and human brucellosis are mainly reported when people or animal knock to the door of clinicians. Serological testing using the Rose Bengal Test (RBT), SAT, TAT, mercaptoethanol test and/or ELISA are generally used for the detection of *Brucella* infections in livestock. Serological test are more rapid and reliable to study the prevalence of brucellosis in a large population. Bangladesh. The epidemiology of *Brucella* spp is believed to be complex and it is influenced by several non-technical and technical phenomena (Nicoletti, 1980). The density of animal populations, the herd size, the type and breed of animal (dairy or beef), the type of husbandry system and other environmental factors are thought to be important determinants of the infection dynamics (Salman and Meyer, 1984).

The present study was undertaken to investigate the seroprevalence of brucellosis in selected areas of Mymensingh and Netrokona district in relation to age, sex, reproductive disorder housing and rearing condition.

MATERIALS AND METHODS

Experimental design

Venous blood samples were randomly and aseptically collected from sheep in Mymensingh and Netrakona District of Bangladesh. In Mymensingh District, blood samples were collected from sheep in Sheep and Goat Farm, Bangladesh Agricultural University (BAU) and its surrounding areas In Netrakona District, sheep blood samples were collected from Netrakona Sadar Veterinary Hospital and several villages of Maska union (Table 1). During sampling information of animal's age, sex, location, pregnancy status, abortion and other reproductive disorder were recorded. Sera were seperated from blood and tested for *Brucella ovis* antibody response. About 102 sheep were selected for epidemiological investigation of brucellosis. Rose Bengal test (RBT) was done to detect the *Brucella* organism, indirect ELISA assay were performed to confirm the detection.

Serological methods

Collection of serum samples

About 5-7 ml of blood was aseptically collected from jugular vein of each sheep with the help of sterile disposable syringe and needle. The syringe was kept undisturbed on a tray for at least 1 hr at room temperature in a slightly inclined position to facilitate clotting and separation of serum. After this period, the clotted blood samples with sera were transferred to a refrigerator and kept overnight at 4^{o}C. Later on, the sera were poured into the separate test tube. The sera were centrifuged at 2,500 rpm for 10 minutes and the cleared sera were transferred to the sterilized labeled eppendorf tube and stored at -20^{o}C until use.

Table 1
Number of sample from different areas

Variables	Category level	Number of observation
Area of sample collection	Sheep and Goat Farm, BAU	15
	Digarkanda	8
	Shesmoar	9
	Kewatkhali	15
	Pilkhana	30
	Veterinary Hosp. Netrakona	15
	Maska Union	10
Gender	Male	25
	Female	77
Age (years)	< 1 year	8
	1 year to 2 years	71
	> 2 years	23
Pregnancy status	Yes	10
	No	53
History of abortion	Yes	9
Client's complain	Anestrous	15
	Failure to concept	62
Housing system	Individual	42
	Mixed	60

Serological tests

Rose Bengal Test (RBT)

The test was performed according to the standard procedure mention in OIE (2004). The test sera sample and control sera were homogenized using a vortex (Shaker). Each serum (30 µl) to be tested was placed on a glass plate circled approximately 2 cm in diameter. Then the vial of antigen was shaked gently and 30 µl of antigen was put beside each of the sera. The antigen and serum were mixed on the plate for exactly 4 minutes, the reading was taken immediately. The result was considered positive when there was any degree of agglutination

Indirect Enzyme Linked Immunosorbent Assay (i-ELISA)

The assay was performed according the protocol provided by the ELISA kit manufacturer (Svanova Biotech, Uppsala, Sweden). Briefly, 100 µl of sample dilution buffer was added to each well microplate. After that 4 µl of positive control serum (Reagent A) and 4 µl of negative control serum (Reagent B) were added to the selected wells coated with *Brucella abortus* antigen. For confirmation control sera were run duplicates. 4 µl of serum sample was added to a selected well

coated with *Brucella abortus* antigen. The plate was shaked thoroughly and sealed the plate/strip and incubated at 37°C for l hr. The plates was rinsed 3 times with PBS-Tween Buffer and filled up the wells at each rinse, emptied the plate by tapping hard to remove all remaining fluid. 100 µl of HRP conjugate (anti-bovine I_gG conjugate) was added to each well and incubated at 37°C for 1 hr. the plate was rinsed again. Then 100 µl substrate solution was added to each well and incubated for 10 minutes at room temperature (18 to 25°C). The reaction was stopped by adding 50 µl of stop solution to each well. The stop solution was added in the same order as the substrate solution was added. The optical density (OD) of the controls and samples was 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.

Calculations of results (percent positivity values -PP) were done as described below:

$$PP = \frac{\text{Test Sample or Neg C (OD)}}{\text{Positive Control (OD)}} \text{ x100}$$

The assay was calibrated against the OIE ELISA Standard sera and standardized against the EU directives 64/432/EEC.

Table 2
Overall seroprevalence of brucellosis in sheep in Mymensingh and Netrakona districts

Study areas	Total number of sera	No. of RBT positive cases (%)	No. of ELISA positive cases (%)	Odds ratio	Overall sero-prevalence
Mymensingh district	77	7 (9.09)	4 (5.19)	1.75	9.80%
Netrakona district	25	3 (12.0)	2 (8.0)	1.5	

Table 3
Prevalence of brucellosis in sheep belonged to different age groups, housing and rearing system in Mymensingh district

	Age			Sex		Housing		Rearing	
Sero prevalence	6 m to < 1 Y	1 Y to 2 Y	> 2 years	Male	Female	individual	Mixed	Individual	Collective
RBT (%)	12.5	8.0	10.52	20.0	8.77	8.0	9.61	13.04	9.25
iELISA (%)	0.0	6.0	5.26	0.0	7.01	4.0	5.76	4.34	5.55

Table 4
Prevalence of brucellosis in sheep belonged to different age groups, housing and rearing system in Netrokona district

	Age			Sex		Housing		Rearing	
Sero prevalence	6 m to < 1 Y	1 Y to 2 Y	> 2 years	Male	Female	individual	Mixed	Individual	Collective
RBT (%)	0.0	10.0	25.0	0.0	15.0	11.11	12.57	20.0	10.0
iELISA(%)	0.0	5.0	25.0	0.0	10.0	11.11	6.25	20.0	5.0

RESULTS AND DISCUSSION

The seroprevalence of brucellosis in two districts (Mymensingh and Netrokaona) of Bangladesh was investigated. Serum samples were collected from 102 sheep of these two different districts tested by RBT and iELISA. The prevalence observed by RBT in Mymensingh was 9.09% and Netrokona 12%. Wherease the prevalence observed by iELISA was 5.19% in Mymensingh and 8% in Netrokona. The overall seroprevalence of brucellosis in sheep was 9.80% (table 2).

In Mymensingh district, prevalence of brucellosis was lowest in case of sheep between 1-2 years (8% by RBT) but the highest prevalence of brucellosis was recorded in case of sheep between 6 months to less than 1year age (12.5% by RBT). In Netrakona district, the lowest prevalence of brucellosis was recorded in sheep belonged 6 months to < 1 year (0.0%) both by RBT and iELISA . Whereas prevalence of brucellosis was highest in case of sheep above 2 years age (25.00 %) both by RBT and iELISA.

Sex related seroprevalence study showed that of in Mymensingh brucellosis in male sheep was recorded as 20.0 % by RBT, and 0% by iELISA. Whereas prevalence of brucellosis in female sheep was recorded as 8.77% by RBT and 7.01% by iELISA. In Netrakona district, the prevalence of brucellosis in male sheep was recorded as 0% by RBT and iELISA. Whereas prevalence of brucellosis in female was recorded as 15.0% by RBT and 10.0% by iELISA. Prevalence of brucellosis in sheep was higher in male than female in Mymensingh. In Netrakona only female were found to be infected with brucellosis.

In this study the seroprevalence of brucellosis in sheep was 9.80% which is higher than the observed seroprevalence of brucellosis at 2% reported by Amin *et al.* (2004), 2.33% by Amin (2003) and 4.84% by Uddin *et al.* (2007). Uddin *et al.* (2007) reported that highest prevalence 8.00% in BAU Farm and 8.32% in Ishwarganj.

In this study it was revealed that, brucellosis of sheep in Netrakona is 1.32 times higher than that of a sheep in Mymensingh indicating that the risk of being infected by brucellosis of sheep in Netrakona is 1.32 times more than that of a sheep in Mymensingh.

In case of age related seroprevalence in Netrakona and Mymensingh, among the three age groups, the highest prevalence of brucellosis was found in above 2 years of age group in Netrakona (25.5%) and in Mymensingh (6.00%) the highest prevalence of brucellosis was found in between 1 to 2 years. Sergeant (1994) found that there was no apparent association between age and serological status, or age and the prevalence. But Ghani *et al.* (1998) found that several epidemiological factors, such as age, sex, breed, lactation number, herd size and living conditions influence the sero-prevalence of brucellosis. In a study by Mudit *et al.* (2005) it was observed that the brucellosis prevalence in goats varied with age as 1.63%, 0.58% and 1.65% of kids, young adults and adults, respectively.

The prevalence of brucellosis in sheep was found to be higher in male (20%) than female 8.77% by RBT in Mymensingh district. This finding was similar to the findings recorded by Sharma *et al.* (2003). In this study, the highest prevalence of brucellosis was found in Netrakona district especially in female with the prevalence of 10.00% detected by ELISA tests, as compared to in Mymensingh district (7.01%).

Seroprevalence of brucellosis among sheep belongs to different housing and rearing systems

In Mymensingh district, the prevalence of brucellosis among sheep kept separately was 8.0 % by RBT and 4.0% was found by iELISA. The sheep kept with other species (cattle and goats) showed the prevalence of 9.61% by RBT and5.76% by iELISA. Whereas the prevalence of brucellosis was 11.11% by RBT and 11.11% by iELISA in Netrakona district in sheep kept separately and the prevalence of brucellosis in sheep kept with other species was 12.57% by RBT and 6.25% by iELISA. However, the prevalence of brucellosis was recorded higher in sheep kept

separately than sheep kept with other species of animal.

The prevalence of brucellosis in collectively reared sheep was 9.25% by RBT and 5.55% by iELISA respectively in Mymensingh district. Whereas the individual rearing sheep showed prevalence of 13.04% by RBT and 4.34% by iELISA. In Netrakona, the prevalence of brucellosis among sheep in collectively rearing system recorded as 10.0% by RBT and 5.0% by iELISA. The prevalence of brucellosis in individually reared sheep was 20.0% by both RBT and iELISA. However, the prevalence was found higher in sheep reared individually than sheep reared collectively.

Table 5
Prevalence of brucellosis in sheep with history of reproductive disorder and pregnancy in Mymensingh district

Sero prevalence	Reproductive disorder			Pregnancy status	
	Abortion	Anestrous	Failure to conception	Pregnant	Non pregnant
RBT (%)	14.28	10.00	9.52	16.66	9.09
iELISA (%)	14.28	10.00	7.14	16.66	6.18

Table 6
Prevalence of brucellosis in sheep with history of reproductive disorder and pregnancy in Netrokona district

Sero prevalence	Reproductive disorder			Pregnancy status	
	Abortion	Anestrous	Failure to conception	Pregnant	Non pregnant
RBT (%)	33.33	0.0	23.07	33.33	20
iELISA (%)	33.33	0.0	15.38	33.33	10

Prevalence of brucellosis in sheep with history of reproductive disorders

The prevalence of brucellosis in aborted sheep was recorded as 14.28% by RBT and 14.28% by iELISA in Mymensingh. Whereas prevalence of brucellosis in sheep with no history of abortion was recorded as 9.30 % by RBT and 6.98% by iELISA in Mymensingh. In Netrakona district the prevalence of brucellosis in sheep with history of abortion was recorded as 33.33 % by RBT and iELISA. The prevalence of brucellosis in sheep without abortion was recorded as 20.0% by RBT and10.0% by iELISA. Based on this data it can be concluded that the prevalence of brucellosis was higher in sheep with history of abortion in Mymensingh and Netrakona districts than sheep with no abortion record.

The prevalence of brucellosis in anestrous sheep was recorded as10.0% by RBT and 10.0% by iELISA respectively in Mymensingh district. The prevalence of brucellosis in sheep with conception failure was recorded as 9.52 % by RBT and 7.14% by iELISA in Mymensingh. No brucellosis case was found in anestrous sheep in Netrakona district. The prevalence of brucellosis in sheep with conception failure was recorded as 23.07 % by RBT and15.38% by iELISA in Netrakona.

Prevalence of brucellosis in pregnant and non-pregnant sheep

The prevalence of brucellosis in pregnant sheep was recorded as 16.66% by RBT and iELISA in Mymensingh district. The prevalence of brucellosis in non-pregnant sheep was recorded as 9.09% by RBT and 6.18% by iELISA in Mymensingh district. In Netrakona, prevalence of brucellosis among pregnant sheep was recorded as 33.30 % by RBT and iELISA. Brucellosis

prevalence among non-pregnant sheep was recorded as 20.0% by RBT and10% by iELISA. Data of this study indicated that in pregnant sheep the prevalence of brucellosis was higher than the non-prenant sheep.

The prevalence of brucellosis was higher in sheep with abortion was (20.0%) as compared to 7.54% in non aborted sheep. Mahajan and Kulshreshtlia (1987) found 74.66% prevalence of brucellosis in aborted sheep and 20.38% prevalence in health sheep.

The prevalence of brucellosis was higher in pregnant sheep (22.22 %) as compared to non-pregnant sheep (7.40%). Amirul Islam (2010) found 12.5% prevalence in pregnant sheep and 4.35% in non-pregnant sheep.

Overall prevalence of brucellosis in sheep was 9.80%. Higher prevalence of brucellosis was recorded in sheep above 2 years old. Prevalence of brucellosis was found to be higher in female as compared to male. Higher prevalence of brucellosis was recorded in sheep with history of abortion. Seroprevalence of brucellosis was in pregnant sheep as compared to non-pregnant.

Prevalence of brucellosis was 1.32 time higher in sheep of Netrakona district as compared to Mymensingh. Seroprevalence of brucellosis was studied by applying RBT and iELISA. The number of positive cases was lower when screened by iELISA compared to RBT. As having limitation of these two tests it further studies for isolation, identification and characterization of *Brucella* organism by molecular approaches are recommended.

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