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SEROPREVALENCE OF BRUCELLOSIS IN SHEEP IN THE GAIBANDHA DISTRICT OF BANGLADESH

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ABSTRACT

Brucellosis is an important zoonoses causing signififant economic loss but is very often neglected in Bangladesh. Therefore, a survey was undertaken to investigate the seroprevalence of brucellosis in sheep of selected areas (Gaibandha sadar and Gobindagonj upazilas) in the Gaibandha districts of Bangladesh. A total of 206 sera samples were collected from sheep and were tested for presence of *Brucella* specific antibody by Rose Bengal Plate Test (RBPT) as screening test and the RBPT positive samples were further confirmed using indirect Enzyme-linked immunosorbent assay (i-ELISA). Information of sheep's age, sex, housing system, pregnancy status, abortion and reproductive disorder were collected using questionnaire. The overall seroprevalence of brucellosis in sheep was recorded as 3.39% in RBPT and 2.91% in i-ELISA. The prevalence of brucellosis in female sheep (3.41%) was higher than male (3.33%). The prevalence of brucellosis in sheep with abortion history was higher (4.34%) than the sheep with no abortion (3.08%). The highest prevalence of brucellosis (4.00%) was found in sheep keeping with others species such as cattle and goat compared to the sheep keeping alone (1.79%). The higher rate (4.59%) of *Brucella* antibody was recorded in sheep of 1-2 years of age. Brucellosis might be an important hinders for sheep production in Bangladesh. The present study will help to develop an appropriate prevention strategy for brucellosis in Bangladesh.

Key Words: Seroprevalence, Brucellosis, Sheep, RBT, I-ELISA

INTRODUCTION

Brucellosis is a major constraint for the development of livestock in Bangladesh. Brucellosis was first detected in cattle (Mia and Islam, 1967), in buffalo (Rahman *et al*., 1997) and in human (Rahman *et al*., 1983). It is widely studied in cattle (Rahman *et al*., 2009, 2006; Amin *et al*., 2004) but limited information could be found in the case of small ruminants such as sheep and goats. However, sheep and goats are playing an important role in the economic well being of the resource-poor farmer. The epidemiology of

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Brucella sp. is believed to be complex and it is influenced by several non-technical and technical phenomena. 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 (Uddin *et al*., 2007a,b). Brucellosis remains a major source of disease in humans and domesticated animals worldwide. Although the prevalence of this disease varies widely from country to country, small ruminant brucellosis is mostly caused by *B. meltensis*. (Redkar *et al*., 2001). *B. ovis* is also an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats. Brucellosis spreads between animals in a herd and the disease is a systemic infection that can involve many organs and tissues. Once the acute period of the disease is over, symptoms of brucellosis are mostly not pathognomonic, and the organism can be chronically located in the supramammary lymphatic nodes and mammary glands of 80% of infected animals. Thus they continue to secrete the *Brucella* organism in their body fluids (Redkar *et al*., 2001).

The brucellosis can have a considerable impact on human and animal health, as well as socioeconomic impacts, especially in which income relies largely on livestock breeding and dairy products. Brucellosis in human beings is caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and can also be transmitted to consumers through raw milk and milk products. Brucellosis has been reported in small ruminants from different parts of the world (Uddin *et al*., 2007b; Bandeg *et al*., 1989; Abd-el-Ghani *et al*., 1983). Brucellosis in cattle, buffalo and human beings has been widely investigated by many investigators (Rahman *et al*., 2006, 1983) but limited research has been done to unravel the seroprevalence of brucellosis in sheep. No study was carried out for brucellosis in Gaibandha in Bangladesh. Therefore, the aim of this study was carried to determine the seroprevalence of brucellosis in sheep in Gaibandha district of Bangladesh.

MATERIALS AND METHODS

Experimental design

Venous blood samples were collected aseptically from randomly selected 206 sheep in Gaibandha sadar and Gobindhagonj upazilas of Gaibandha districts of Bangladesh. During sampling, information on age, sex, breed, pregnancy status, reproductive problems such as repeat breeding, previous abortion and retention of placenta were recorded using questionnaire. After collection of data, about 5-7 ml of blood was collected aseptically from each of the randomly selected Black Bengal goats. All the blood samples were processed for sera preparation.

Serological tests

Rose Bengal plate test (RBPT) test were used for the diagnosis of brucellosis as screening test and the animals found positive in RBPT were further confirmed by i-ELISA test.

Rose bengal plate test (RBPT)

The RBPT was performed according to the procedure as described by OIE (2004) and Uddin *et al.* (2007a,b). The test serum samples and *Brucella* antigen (William James House, Cowley Rd. Cambridge, CB4 0WX, UK) were kept one hour in room temperature before beginning of the test. A total of 30 µℓ of each serum to be tested was placed on a glass plate circled, approximately 2 ㎝ in diameter. Then the vial of antigen was shacked gently and 30 µℓ of antigen was 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 80-100 rpm for 4 minutes and the reading was taken immediately. Any agglutination or precipitation was considered as positive, whereas no reaction (negative) was indicated as the absence of *Brucella* antigen in the sera. The positive and negative reactions are given in Fig. 1.

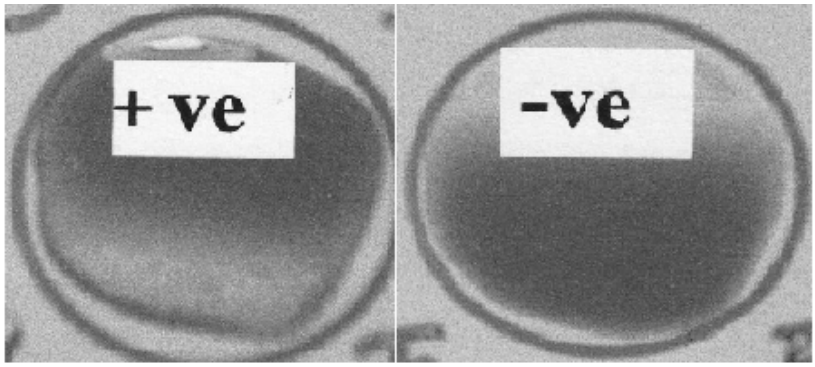

Fig. 1. Positive (+ve) and negative reaction of RBPT

Indirect enzyme linked Immunosorbent assay (i-ELISA)

The assay was performed according to the manufacturer's instructions (Svanova Biotech AB, art.No.10-2700-10, SE-751 83Uppasala, Sweden). First of all, the PBS-Tween Buffer and conjugate lyophilized horse radish peroxides (HRPO) conjugate was reconstituted immediately before use to perform i-ELISA. In brief, all reagent supplied by manufacturer was equilibrated to room temperature at 18 to 25^{o}C before use. A volume of 100µℓ of sample dilution buffer was added to each well by multichannel micropipette that would be used for serum samples and serum controls. After that 4 µℓ of positive control serum (Reagent A) and 4µℓ of negative control serum (Reagent B) were added respectively, to selected wells coated with *B. melitensis* antigen. For conformation purposes, the experiment was run with the control sera in duplicates. A volume of 4µℓ of sera samples were added to the selected well coated with *B. melitensis* antigen. For conformation purposes, the samples were also run in duplicates. The plate was shacked thoroughly and sealed the plate/strip and incubated at 37 ^{o} for one hour. The plates ware rinsed three times with PBST buffer and filled in the wells at each rinse, emptied the plate and tapped hard to remove all remains of fluid. Then 100µℓ of HRP conjugate was added to each well

and incubated at 37° for one hour followed by a rinsing of the plate. Then 100µℓ substrate solutions was added to each well and incubated for 10 minutes at room temperature. The reaction was stopped by adding 50µℓ of stop solution to each well and mixed thoroughly. The stop solution was added in the same ordered as the substrate solution was added. The optical density (OD) of the controls and samples was measured at 450㎚ in a microplate photometer. The OD was measured within 15 minutes after the addition of stop solution to prevent fluctuation in OD values. The positive and negative reactions are given in Fig. 2. The percent positivity values (PP) were calculated using the formula according to the manufacturer's guideline:

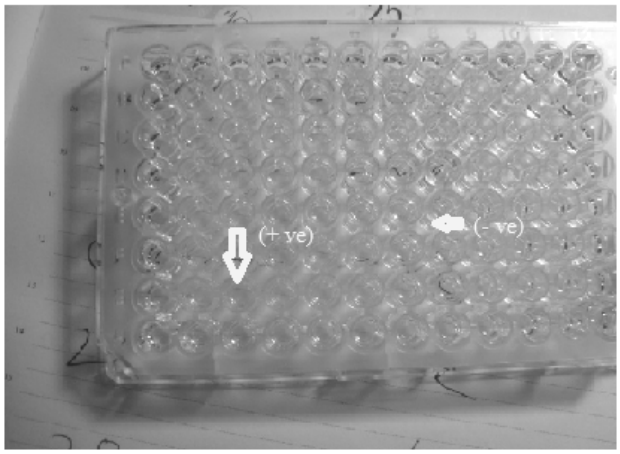

Fig. 2. Positive (+ve) and negative reaction of i-ELISA

Statistical analysis

The Chi-square test was employed to find out the significant relationship between the prevalence of brucellosis demographic variables (age, sex, species, breed etc) by using SPSS version 17.0.

RESULTS

Out of 206 samples, 56 and 150 samples were collected from Gaibandha sadar and Gobindhagonj upazilla of Gaibandha district, respectively. The overall prevalence of brucellosis in sheep was 3.39% (Table 1). Sex wise seroprevalence of brucellosis revealed that prevalence in male was 3.33%, whereas in case of female it was recorded as 3.41% which implies that prevalence of brucellosis in female sheep was higher than male sheep. However, the occurrence of brucellosis had no significant relationship with sex of sheep (Table 1). When age-wise seroprevalence of brucellosis was calculated, it was found that prevalence was lowest in sheep of 6 months to 1 years of age (2.13%). The seroprevalence of brucellosis was recorded in sheep relatively higher (4.59%) in 1 year to 2 years age group, compared to 2 years to 3 years age group (2.86%), and no positive case was found on the age group above 3 years.

Table 1. Seroprevalence of brucellosis and its association with different factors in sheep of Gaibandha district in Bangladesh

	Number of sera samples tested	Number of positive samples in RBPT	Number of positive samples in i-ELISA	Prevalence (%)	Level of significance
Sex					
Male	30	1	1	3.33	NS
Female	176	6	5	3.41	
Total	206	7	6	3.39	
Age of animals					
6 months to 1 year	47	1	1	2.13	
1 year to 2 years	109	4	4	4.59	
2 year to 3 years	35	1	1	2.86	*
Above 3 years	15	0	0	0	
Pregnancy status					
Pregnant	50	1	1	2	NS
Non-pregnant	126	5	4	3.97	
Types of reproductive disorders					
Anestrous	44	1	1	2.27	
Failure of conception	52	3	2	5.77	
Retention of placenta	32	1	1	3.12	
Dystocia	5	0	0	0	NS
Repeat breeder	27	1	1	3.70	
Others (such as whitish discharge)	16	0	0	0	
Abortion history					
Abortion	46	2	2	4.34	NS
No abortion	130	4	3	3.08	
Keeping system					
Keeping separately	56	1	1	1.79	
Keeping with others species	150	6	5	4.00	*
Floor type					
Concrete floor	41	1	1	2.43	NS
Earthen floor	165	6	5	3.64	

NS = Not significant, *Significant at 5% level of probability

Statistically, there is existed a significant ($P<0.05$) relationship between age of sheep and the prevalence of brucellosis (Table 1). Although, the prevalence of brucellosis had no significant relationship with reproductive problems of sheep statistically, there was variation for prevalence between sheep with different reproductive problem could be found (Table 1). Prevalence of brucellosis in sheep with history of anestrous (2.27%),

failure of conception (5.77%), retention of placenta (3.12%) and repeat breeder (3.7%) are detected. The prevalence was higher in sheep with history of abortion (4.34%) compared to the sheep with no abortion history (3.08%). Keeping system of animals means whether the sheep kept separately or kept with other species such as cattle and goat have significant effect on the prevalence of brucellosis in sheep ($P<0.05$). The sheep kept separately had lower prevalence (1.79%) compared to the sheep kept with other livestock (4.0%) (Table1). Higher prevalence could be found in the sheep kept in the house with earthen floor (3.64%) compared to the sheep kept in house with concrete floor (2.43%) (Table 1).

DISCUSSION

Brucellosis remains as a major zoonosis worldwide (WHO, 1986). Although many countries have eradicated *B. abortus* from cattle, in some areas it has emerged as a cause of infection in this species as well as in sheep and goats. The importance of brucellosis was primarily due to its public health significance and economic loss to the animal industry (WHO, 1971). Bangladesh has been reported as an endemic country for brucellosis because of a considerable number of human and animal populations are exposed to the infection each year (Rahman *et al*., 2006). Definitive diagnosis of brucellosis can be accomplished only through the direct demonstration and identification of the causative agent(s) by culture and isolation procedures (Orduña *et al*., 2000).

The present investigation revealed that the overall seroprevalence of brucellosis in sheep was 3.39% which is higher than the overall seroprevalence of bruceIlosis, 2% reported by Amin *et al*. (2004). However, these results are close to the result of Abd-el-Ghani *et al*. (1983) reported 2.97% brucellosis in sheep. This finding is in agreement with Rahman *et al*. (2006) who reported animal/individual-level seroprevalence of brucellosis in cattle is 2.4%-18:4% while the herd-level seroprevalence in cattle is 62.5%. It is difficult to compare these results with other in Bangladesh because there are limited studies on brucellosis in sheep. Osman and Adlan (1987) reported 0.27% brucellosis of sheep in Saudi Arabia. Bandeg *et al*. (1989) reported brucellosis infection 3.2% in Merino sheep in Kashmi. Burriel *et al*. (2002) found 16.8% of sheep were positive to *Brucella* infection in Greece. Prevalence rate of 1.7% in sheep and 1.5% in goats in Sudan (Abdalla, 1966); 6.01% in sheep and goats in Kenya (Waghela, 1976); 3.8% in goats and 1.4% in sheep in Eritrea (Omer *et al*., 2000); 4% in goats and 1% in sheep in eastern Sudan (El-Ansary *et al*., 2001). From 255 sheep and 289 goats slaughtered at an abattoir of New Delhi India, brucellosis was diagnosed in 9.02%, 4.31%, 27.45% and 10.95% sheep and 1.73%, 1.38%, 7.27% and 18.34% goats using RBPT, Standard Tube Agglutination Test (STAT), Complement Fixation Test (CFT) and dot - ELISA, respectively.

In case of age related seroprevalence in Gaibandha district, among the four age groups, the highest prevalence of brucellosis (4.59%) was found in 1 to 2 years of age group. Sergeant (1994) found that there was no apparent association between age and serological status, or age and the prevalence. But Ghani *et al*. (1998) stated that several

epidemiological factors, such as age, sex, breed, lactation number, herd size and living conditions influence the sero-prevalence of bruccllosis which is in agreement with our findings. It might explain because within this period at 1 to 2 years of age, the sheep is very active in reproduction. The prevalence of brucellosis in sheep was found to be higher (3.41%) in female than male (3.33%) which is similar to the findings recorded by Sharma *et al*. (2003). The prevalence of brucellosis was higher in sheep with abortion (4.34%) as compared to non aborted sheep. Mahajan and Kulshreshtha (1987) found 56 positive cases out of 75 aborted sheep and 76 positive cases out of 373 healthy sheep. It could be explained because *Brucella* is one of the main bacterial causal agent causing abortion in sheep. In this study, higher prevalence of brucellosis was found in sheep keeping with cattle, buffalo and goat (4.00%) as compared to separate keeping system (1.79%). Omer *et al*. (2000) found 8.2% prevalence in individually reared sheep and 35.9% in herd sheep. The prevalence was higher in sheep housed on earthen floor (3.64%) compared to the sheep housed on concrete floor (2.43%). The earthen floor might be more suitable for the habitation and growing of bacteria and other microorganism compared to the concrete floor because the earthen floor is mostly damp and dirtier. Notably, it could be seen there are some discrepancies between the RBPT and i-ELISA in this study which is common in case of serological test because different serological tests such as RBPT, CFT, STAT and i-ELISA varies in sensitivity and specificity (Rahman *et al*., 2010).

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