

## SEROLOGICAL PREVALENCE OF OVINE AND CAPRINE BRUCELLOSIS IN BANGLADESH

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### ABSTRACT

Brucellosis is considered to be the most widespread zoonosis throughout the world. It has a serious implication on human health as well as on the economic development in a developing country like Bangladesh. The objective of the present study was to determine the seroprevalence and to delineate the risk factors for *Brucella* seropositivity in small ruminants in Mymensingh district of Bangladesh. In the present study, serum samples were collected from a total of 2456 small ruminants (1710 goat and 746 sheep) from 13 upazilla of Mymensingh district. The data related to age, sex and location were also collected using a questionnaire. Serum samples were screened using Rose Bengal Test (RBT) and Enzyme Linked Immunosorbent Assay (ELISA). Seroprevalence of brucellosis was 9.53% in goats and 9.92% in sheep on RBT test. In goat, the highest *Brucella* antibody was observed in Mymensingh sadar upazilla (13%) followed by Dhobaura upazilla (12.9%). On the other hand, highest ovine *Brucella* antibody observed in Haluaghat upazilla (13.04%) followed by Mymensingh sadar (12.5%). The prevalence was more in adults (55.2% in goats and 57% in sheep) than young (8.6% in goat and 8.1% in sheep) and more in female goats (41.1%) and sheep (39.2%) than male goats (14.1%) and sheep (18%). ELISA test showed 33.70% (31 out of 92 RBT positive samples) positive reaction of total RBT positive reactors. The result of this study can be useful to initiate and establish a program for controlling and prevention through test and slaughter, culling of infected animal from flock and vaccination.

**Key words:** Brucellosis, RBT, iELISA, goat, sheep, prevalence, Mymensingh.

### INTRODUCTION

Caprine and ovine brucellosis caused by zoonotic gram-negative, coccobacillae, facultative intracellular bacteria which belong to the genus *Brucella*, comprising at least 10 species which includes *Brucella melitensis* one of three biovars (biovars 1, 2 and 3) and *B. ovis* as well as many other species is an economically important cause of abortion in small ruminants causes significant losses from decreased productivity and lost trade in much of the developing world. The natural reservoirs of the species *B. melitensis* are basically goats and sheep but also cattle and swine. However, *B. ovis* is primarily afflicting sheep (Lopes *et al.*, 2010; CFSPH, 2009; Olsen and Palmer, 2014). Brucellosis has a considerable impact on human and animal health as well as a socioeconomic impact, and especially in rural areas that largely rely on livestock and in developing countries still considered as the most serious and devastating zoonotic disease (Al-Majali, 2005; Al-Majali *et al.*, 2007; Rahman *et al.*, 2015). In animals, brucellosis causes severe economic losses as result of stormy abortions or reproductive failure, sterility and reduced milk production rates, besides to that, it adds to the burden shouldered by the farmers; the costs of control and management as well as reduces the Foreign Exchange Earnings (FEE) by denying exportation of sheep to international markets (McDermott, 2013; Angara and Ali, 2014). However, the causative agent has a very low infectious dose (10 organisms of *B. melitensis*) are sufficient to cause an infection in man (Lopes *et al.*, 2010).

The goat has played an important role in traditional Bengali society among the Asiatic countries, a tropical agro-based developing country economically and culturally, possesses the third largest repository of goats, with a population of more than 14.8 million heads, 57% of total livestock in Bangladesh, according to the FAO (WHO, 2006; Banglapedia, 2016). Total sheep population has estimated of 1.9 million. In Bangladesh about 83.9 percent

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of total households own livestock among which 0.9% are goat and sheep (Banglapedia, 2016). Each year 127,000 MT representing 25% of total red meat in Bangladesh are produced from goat and more than 98% of goats are owned by the small, marginal and landless farmers in the villages (Bangladesh Economic Review, 2011). Important factors that contribute to the spread of brucellosis in goat and sheep include: farming system and practices, farm sanitation, livestock movement, mixing and trading of animals, and sharing of grazing grounds and watering points (Kabagambe *et al.*, 2001; Kadohira, 1997; Omer, 2000).

A lot of undiagnosed cases of abortion, stillbirth and retained placenta which are thought to be down to brucellosis and these have a significant impact on the development of livestock in Bangladesh (Rahman *et al.*, 2011). Mammary gland may also be infected in sheep and goats resulting in mastitis, a commonly observed feature of caprine brucellosis compared with bovine brucellosis characterized by multinodular firmness with watery, clotted milk (Cutler *et al.*, 2005). Prolonged excretion of organisms in milk may occur in goats but less so in sheep (Poester *et al.*, 2013). *Brucella 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 (Jacques, 1998).

Screening of brucellosis in sheep and goat using the RBT and iELISA are generally used for the detection of *Brucella* antibody in serum collected from suspected animals. ELISA have been evaluated for many years for their ability to detect serum antibodies to brucellosis in domestic animals. ELISA for the diagnosis of brucellosis has several advantages when compared with other tests. First, it directly identifies a specific antibody. Second, it is more sensitive than other agglutination tests and thus has the potential to detect infected animals. Third, ELISA results provide an epidemiological tool for investigating the infection status of flocks in places where vaccination has never been practiced, like Bangladesh (Rahman, 2005). Indirect ELISA test were used along with RBT for more specific result to avoid false positive result in RBT. These can be used for rapid test for identification of brucellosis infection in small ruminants and introduce preventive measures through initiation of vaccination.

## MATERIALS AND METHODS

### Sample collection

To determine the seroprevalence of caprine and ovine brucellosis in Mymensingh district, a survey plan was designed in both longitudinal and cross-sectional dimension covering all upazilla of Mymensingh district. The research was carried out during the period from January to November 2016. Samples were collected from randomly selected native goat and sheep in Mymensingh Sadar (200, 120), Muktagachha (95, 39), Fulbaria (187, 53), Trishal (210, 62), Valuka (120, 42), Tarakanda (72, 35), Gauripur (112, 84), Nandai (55, 80), Ishwarganj (28, 81), Gaffargoa (123, 20), Dhobaura (163, 35), Fulpur (150,49), Haluaghat (195,46). About 5 ml blood was collected from jugular vein of each of the selected goat and sheep 1710 and 746 respectively in separate sterilized test tubes (a total of 2456 sera samples) and kept in refrigerator overnight. The test tube was further refrigerated at 4-8°C overnight. Later, the sera were separated from blood clots and centrifuged at 2500 rpm for 10 min to obtain clear sera free from blood cells. Finally, sera were transferred into a sterilized Eppendorf tube and stored at -20 °C until used. The data related to age, sex and location were also collected on the sampling day. Different serological tests, Rose Bengal Plate Test (RBPT) and indirect Enzyme-Linked Immunosorbent Assay (iELISA), were performed to determine the true prevalence of brucellosis.

### Rose Bengal Test (RBT)

Approximate number of processed test sample (serum), sufficient antigen, positive and negative control sera for a day's testing were removed from refrigeration and kept for adjusting at room temperature (approx. 22°C). 30 µl of each serum to be tested was placed on a fine plastic plate circled approximately 1.5 cm in diameter. Then the vial of antigens was shaken gently and 30 µl of antigen put beside each of the sera. The antigen and serum was mixed on the plate with a stirrer and spread over the area enclosed by the circle. Then the plate was placed on a mechanical rotator at 90-100 rpm for 3-5 minutes and the reading was taken immediately. Any agglutination or precipitation was considered as positive, whereas no reaction indicated the absence of *Brucella* antigen in the sera (Figure 4).

**Indirect Enzyme-Linked Immunosorbent Assay (iELISA)**

RBT positive sera was screened with indirect Enzyme-Linked Immunosorbent Assay (iELISA) according the protocol and supplience provided by the ELISA kit (Svanova Biotech AB, Sweden) as per manufacturer instruction and reading was estimated by automated ELISA reader (Figure 5).

**Data analysis**

The data was recorded on excel and were analysed by using SPSS software version 20 (SPSS, Inc., Chicago, IL, USA).



Figure 1. Retention of placenta in a goat

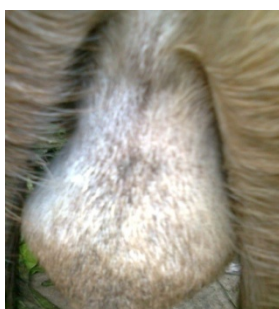


Figure 2. Orchitis in buck



Figure 3. Aborted fetus in a goats



Figure 4. Rose Bangal Test (RBT). Arrows are indicating the positive cases.

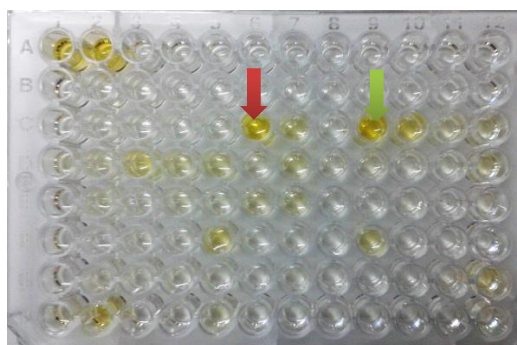


Figure 5. iELISA test result of positive (indicating arrows) RBT serum

**RESULTS AND DISCUSSION**

The positive reactor by Rose Bangal Test (RBT) in goat and sheep were 9.53% (163/1710) and 9.92% (74/746) respectively. Upazilla wise prevalence were estimated of goat and sheep (Table 1). The highest caprine *Brucella* antibody was observed in Mymensingh sadar upazilla (13%) followed by the upazilla Dhobaura (12.9%) and highest ovine *Brucella* antibody observed in upazilla Haluaghat (13.04%) followed by Mymensingh sadar (12.5%). In relation to age and sex, adult goats (55.2%), sheep (57%) were more positive than young (8.6%) & (8.1%) respectively and female goats (41.1%), sheep (39.2%) were more susceptible than male goats (14.1%) & sheep (18%). ELISA test showed 33.70% of RBT reactors had shown positive reaction (Table 2).

Table 1. Upazila wise distribution of prevalence of sheep and goat brucellosis (RBT)

Name of Upazilla	Goat				Sheep			
	Negative	Positive	Total no of test	Prevalence (%)	Negative	Positive	Total no of test	Prevalence (%)
Mymensingh Sadar	174	26	200	13	105	15	120	12.5
Muktagachha	87	8	95	8.4	35	4	39	10.2
Fulbaria	175	12	187	6.4	48	5	53	9.4
Trishal	186	24	210	11.4	55	7	62	11.3
Valuka	107	13	120	10.8	38	4	42	9.5
Tarakanda	70	2	72	2.7	35	0	35	0
Gauripur	103	9	112	8.0	75	9	84	10.7
Nandail	54	1	55	1.8	72	8	80	10
Ishwarganj	28	0	28	0	74	7	81	8.6
Gaffargoan	114	9	123	7.3	20	0	20	0
Dhobaura	142	21	163	12.9	31	4	35	11.4
Fulpur	136	14	150	9.3	44	5	49	10.2
Haluaghat	171	24	195	12.3	40	6	46	13.04
Grand Total	1547	163	1710	9.53	672	74	746	9.92

Brucellosis is a global zoonotic disease, associated with significant morbidity that can lead to increase rate of spontaneous abortion and infertility in livestock and widely distributed throughout the developing world, including Bangladesh, considered to be a serious problem in atleast 86 countries (Hamdy and Amin, 2002; Samad, 2008). The prevalence of caprine and ovine brucellosis in 13 upazillas of Mymensingh district was estimated based on RBT and iELISA. About 1710 and 746 goat and sheep samples were collected respectively based on questionnaire among the flock with any history of abortion and stillbirth, retained placenta and poor conception rate (Figure 1,2,3). Overall prevalence observed by RBT in goat and sheep was 9.53% and 9.92% among which ranged 0% - 13% Ishwarganj and Mymensingh sadar upazilla and 0% - 13.04% Tarakanda, Gaffargoan and Haluaghat respectively. So, 9.53% goat and 9.92% sheep could be infected with brucellosis which may play a great role in intra flock or other means of transmission among them or between man and animals. The highest prevalence in goat was recorded in Mymensingh sadar followed by Dhobaura upazilla as well as in sheep was in Haluaghat followed by Mymensingh sadar were 13%, 12.9%, 13.04% and 12.5% respectively.

Table 2. Age and sex-wise distribution of prevalence of caprine and ovine brucellosis

Age Distribution	Age-wise total				Sex							
	Positive		Prevalence (%)		Male				Female			
	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep
< 6 mon*	14	6	8.6	8.1	3	1	1.8	1.4	11	5	6.7	7
>6 mon - 1yr*	21	9	13	12.2	5	2	3.06	2.7	16	7	9.8	10
>1 - 2yr	38	17	23.3	23	9	7	5.5	9.5	29	10	18	13.5
> 2 - 5yr	90	42	55.2	57	23	13	14.1	18	67	29	41.1	39.2
Grand total	163	74			40	23	24.5	31.08	123	51	75.5	68.91

\*mon= months, yr= year(s)

The age wise distribution estimated prevalence in goat and sheep was higher in above 2 to 5 years old group than the younger and 55.2% and 57% respectively. According to sex wise distribution, prevalence was estimated

higher in female (75.5% and 68.91%) than the male (24.5% and 31.08%) in goat and sheep respectively. Female in above 2 to 5 years group animals were estimated higher prevalence (41.1% and 39.2%) than male above 2 to 5 years group (14.1% and 18%). In both cases, female were found to be more susceptible than male. So, female might have act as reservoir for brucellosis. The total RBT positive samples were performed indirect enzyme linked immunosorbent assay (iELISA) which supported 33.70% (31/92) of RBT. The prevalence and severity of the infection may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factors (Amin *et al.*, 2005). Serological test might be a means for identification of brucellosis across the country and measures could have taken to initiate and establish a program for controlling and prevention through test and slaughter, culling of infected animal from flock and vaccination.

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