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RISK FACTORS ASSOCIATED WITH PREVALENCE OF BRUCELLOSIS IN BLACK BENGAL GOATS IN BANGLADESH

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ABSTRACT

A total of 242 milk and 208 blood samples of goat were collected from three organized goat farms and surrounding rural areas of Bangladesh Agricultural University to determine the prevalence and associated risk factors of brucellosis in Black Bengal goats during the period from December 2008 to September 2009. Milk samples were screened by Milk Ring Test (MRT) and serum samples by Rose Bengal test (RBT) and Micro Agglutination Test (MAT) for detection of brucella specific antibody in milk and blood respectively. The overall prevalence was recorded as 13.64% in milk by MRT; 3.85% and 3.37% in serum by RBT and MAT respectively. About 21.21(7/33) % and18.18 (6/33) % of MRT positive goat showed positive reactions in RBT and MAT respectively. Does aged up to 4 years had lower prevalence (3.70%) of brucellosis than those aged over 4 years (12.50%). About 2.1 (odds ratio, OR = 2.1; 95% CI: 1.21- 4.53) and 47.1(OR = 47.1; 95% CI: 5.3- 416.6) folds increased odds of seropositivity of brucellosis were observed in aborted and placental retention cases respectively. Significantly (p<0.05) higher prevalence of brucellosis was recorded at late lactation stage (17.94%) than those were in mid (16%) and early lactation stage (11.76%). A significantly higher odds of seropositivity of brucellosis was observed in does (OR = 23; 95% CI: 3.08- 173.62). About 7 folds (OR = 6.8; 95% CI: 1.13- 5.32) increased odds of seropositivity was observed in pregnant does.

Key words: prevalence, risk factors, brucellosis, Black Bengal goats

INTRODUCTION

Brucellosis is a global zoonotic disease, associated with significant morbidity that can lead to increase rate of spontaneous abortion and infertility in livestock (Samad, 2008). The disease is widely distributed throughout the developing world, including Bangladesh, considered to be a serious problem in at least 86 countries (Hamdy and Amin, 2002). The epidemiology of brucellosis is believed to be complex and it is influenced by several non-technical phenomenons. Several researchers have extensively reviewed the factors associated with *Brucella* infections in animals and they have classified each variable into one of three categories: which are related to the characteristics of the animal populations, the styles of management and the biology of the disease. Gram negative intracellular bacterium *Brucella melitensis* is the main etiological agent of brucellosis in small ruminants and it can be responsible for bovine brucellosis in some areas (Corbel, 1997) and it is the most important and pathogenic *Brucella* sp. for humans causing clinically apparent human brucellosis (Samad, 2008). These organisms localize in the supramammary lymph nodes and mammary glands in 80% of infected animals which continue to excrete these pathogens in their milk throughout lives and have a significant role in the epidemiology of the disease (Hamdy and Amin, 2002). There is no effective or approved *Brucella* vaccine to be used in human, and therefore the control of the disease in animal reservoirs is paramount for suppression of human disease (Gupta *et al.,* 2006).

The diagnosis of brucellosis in dairy goats involves either the isolation of *Brucella* from milk sample or the detection of antibody in serum or milk. Detection of *Br. melitensis* antibody in individuals of sheep or goat flock is considered to be adequate for control measures to be initiated. Therefore, a particularly sensitive method of detecting *Br. melitensis*-antibodies can be more useful than a specific method, such as isolation of the causative agent, which should be used when an eradication programme reaches its final stages (Burriel *et al.,* 2004). Review of inland literature revealed that the seroprevalence of brucellosis in different livestock species (Samad 2000; Rahman *et al.*, 2006) and human (Muhammad *et al.,* 2010) have been reported from Bangladesh but the works on the epidemiological aspects are very limited in Bangladesh. Therefore, this study was conducted to determine the seroprevalence of brucellosis in Black Bengal goats and to evaluate the risk factors associated with the seropositivity for brucellosis in goats.

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MATERIALS AND METHODS

This cross-sectional study was conducted on a total 242 milk samples collected from apparently healthy Black Bengal goats of three organized goat farms viz. Babul's Farm (n=11), Savar Goat Development Farm (n=89), Rajshahi Goat Development Farm (n=116) and goats brought to the BAU Veterinary clinic (n=26) for treatment. A total of 208 blood sample comprises of 178 from lactating does used for milk sampling and 30 from bucks (10 from SGDF and 20 from RGDF) were also included for this study. This study was conducted over the period from December 2008 to September 2009.

Survey design and sampling

The herd size of Babul's Farm (BF), Savar Goat Development Farm (SGDF) and Rajshahi Goat Development Farm (RGDF) were 42, 1230, 1780 respectively, of which 11(26.19%), 356 (28.94%) and 464(26.07%) goats were lactating respectively. For convenience all lactating does of BF (n=11) and 25% of the lactating does of both SGDF (n=89) and RGDF (n=116) were randomly selected for milk sampling and 26 lactating does registered to the BAU Veterinary clinic for treatment during the study period also were included for milk sampling. Milk samples were collected by the farm attendants after proper disinfection of the udder and teat with 70% ethanol. One to two drops of milk were discarded and then 10 ml of milk were taken from each halves and kept in sterilized test tubes labelled with (L or R). Blood samples were collected from the jugular vein of goats into clean and labeled venoject tubes and allowed to clot. The sera were separated from the collected blood and stored at -20°C until tested. A pre-tested questionnaire was used to collect animal and herd level information at the time of sampling.

Milk Ring Test (MRT)

Milk Ring Test (MRT) was performed by following the manufacturer's instruction. In brief, antigen was kept 1 hour at room temperature (18-23˚C) before the beginning of the test. After proper mixing, 1.0 ml of milk sample to be tested was taken and 50 µl of MRT antigen added in each tube. The milk and MRT reagent was mixed with vortex and incubated for 1 hour at +37°C and then between +2˚C to +8˚C for 18-20 hours. The result was read as positive if the ring of cream equally or more coloured than the underlying milk and as negative if the ring of cream less coloured than the underlying milk.

Rose Bengal Test (RBT)

RBT was performed as described by Alton *et al.* (1988). Briefly, sufficient antigen, test sera, positive and negative control sera for a day's testing were separated from refrigeration and brought to room temperature (22±4°C). Equal volumes (30µl) of serum and antigen (concentrated suspension of *B. abortus* biotype 1 (Weybridge 99); Institut Pourquier, France) were mixed and rotated on a glass plate for 4 minutes. Any noticeable agglutination after this delay was considered positive.

Micro Agglutination Test (MAT)

MAT was performed with EDTA as described by Garin *et al.* (1985). The antigen used was *B. abortus* biotype 1 (Weybridge 99) (Synbiotics Europe, France). One hundred and sixty eight micro litre of SAW buffer in the first well and 100 µl in the second and the third wells were added in 96-well microtitre plate. Thirty two microlitre of serum was added in the first well (Dilution 1:6.25). After proper mixing of diluent and serum, 100 µl from first well transferred to the second well (1:12.5). In the same way 100 µl was transferred from second to the third well (dilution 1:25) and 100µl discarded from the third well. Then in each well 100 µl of standardized SAW antigen was added giving the serial serum dilutions of 1:12.5; 1:25 and 1:50.The plates were agitated and incubated at 37°C for 20-24 h. Reading was done on the basis of degree of agglutination and expressed in international units (iu). Any serum with an antibody titre greater than or equal to 30 iu/ml, as prescribed by the EU (Shey-Njila *et al.*, 2005), was considered positive.

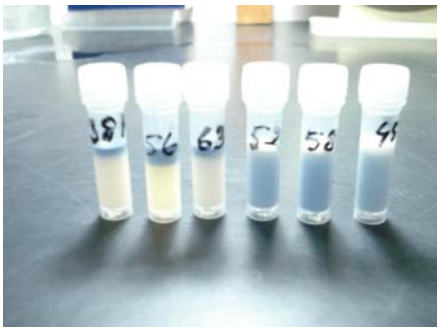

Fig.1 Results of Milk Ring Test (MRT) with caprine milk showing positive reaction (left three) and negative reaction (right three)

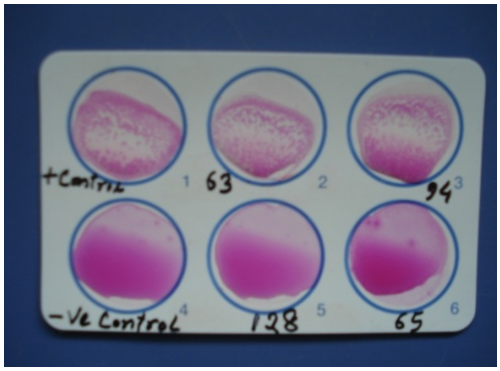

Fig. 2. Results of RBT using serum sample showing definite clumping (63 & 94) indicating positive reaction and no clumping (128 & 65) indicating negative reaction

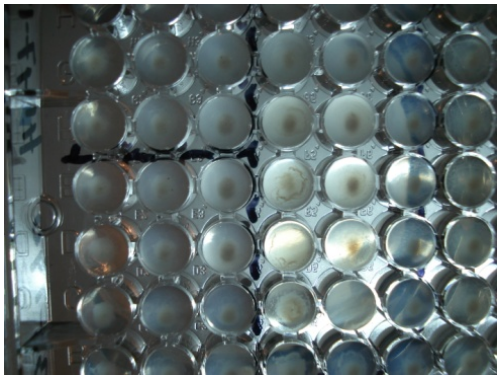

Fig. 3. Results of MAT using serum sample showing agglutination at button of the pointed wells indicating positive reaction.

Statistical analysis

To determine the potential risk factors, goats were considered positive if they showed positive reaction in at least one serological test. Univariate analysis was done using the $\chi 2$ test in using R® (The R Foundation for Statistical Computing, Vienna, Austria). A significance level of 5% was used.

RESULTS AND DISCUSSION

In *Brucella* infected goats, persistent infection of the mammary gland and supramammary lymph nodes is common with constant or intermittent excretion of the bacteria in the milk during the subsequent lactation. This constitutes a serious human health hazard due to the habit of eating raw goat milk at rural areas where man and goat remain close together (Alton, 1985). Therefore this study was carried out on goat milk along with serum to determine the prevalence of brucellosis along with factors associated with seropositivity of brucellosis in goats.

The overall prevalence of *Brucella* antibody in milk was recorded in this study as 13.64% by MRT. A relatively lower prevalence of brucellosis using MRT was reported by Abu El-Razik *et al.* (2007) who reported 8.5% prevalence of *Brucella* reactor by MRT in goat milk. This relatively low prevalence may be due to modification of MRT protocol which may increase specificity of the test. The MRT we used in goat milk is designed for cows milk. It should not be used solely on goat milk to detect brucellosis as it may produce false positive reactions (Shimi and Tabatabai, 1981). In this study a comparison between milk ring test and serological

tests were made. About 21.21(7/33) % and18.18 (6/33) % of MRT positive goat showed positive reactions in RBT and MAT respectively. These findings reveal that higher prevalence of brucella antibody was found in milk sample (13.64%) than that of serum samples as 3.85% by RBT and 3.37% by MAT. Similar observation was also made by Morgan *et al.* (1978). Although El-Loly and Gazi, (2002) reported that goat milk antibody levels reflect the serological status of the animal and can safely be considered serologically positive or negative for brucellosis. Due to difference between the physiologic properties of goat and cow milk, the milk ring test does not perform well in goat samples (Mikolon *et al.,* 1998, OIE, 2000 and FAO, 2003). But modification of MRT by adding 300 µl brucella negative cow cream in 1.0 ml of caprine milk sample may increase the performances as practiced by Abu El-Razik *et al.* (2007).

The distribution of brucellosis detected by MRT was represented in Table 2. Does having age group of ≤ 4 years had lower prevalence (12.44%) than those of more than 4 years (24%). This is supported by the findings of Solorio-Rivera *et al.* (2007) who reported a significantly ($p<0.2$) higher seroprevalence of brucellosis in goat aged at >36 months (12%), followed by aged between 24-36 months (11%) and aged ≤ 24 months (6%). Similar findings were also reported by Muma *et al.* (2006) in Zambia.

Factors significantly associated with brucellosis are shown in Table 1. About 2.1 (odds ratio, OR = 2.1; 95% CI: 1.21 – 4.53) and 47.1(OR = 47.1; 95% CI: 5.3 – 416.6) folds increased odds of seropositivity observed in aborted and placental retention cases respectively. In this study, significantly ($p<0.05$) higher prevalence of brucellosis was recorded at late lactation stage (17.94%) than that of mid lactation (16%) and early lactation stage (11.76%). Statistically insignificant higher (13.89%) prevalence of brucellosis were observed in does reared under farming condition than those reared under rural condition (11.53%). A significantly higher odds of seropositivity was observed in does (OR = 23; 95% CI: 3.08, 173.62). About 7 folds (OR = 6.8; 95% CI: 1.13 – 5.32) increased odds of seropositivity was observed in pregnant does.

Overall seroprevalence of brucellosis was recorded 3.85% and 3.37% in Black Bengal goats by RBT and MAT respectively. This finding is comparable with the report of Lopes *et al.* (2010) who reported the seroprevalence of brucellosis in goat as 2.19% by RBT. However, higher seroprevalence (30.76%) was reported by Junaidu *et al.* (2008).

Factors associated with seroprevalence of brucellosis using RBT and MAT is presented in Table 3. A statistically insignificant higher prevalence of brucellosis was found in goat aged above 4 years (12.50%) than that aged below 4 years (3.70%). Similar findings were also reported by Nahar and Ahmed (2009) and Solorio-Rivera *et al.* (2007). Goats reared under farming condition had lower (4.24%) prevalence than those of reared under rural condition (7.69%) but not statistically significant. This relatively higher prevalence of brucellosis in rural condition may be due to small size of the sample. In the present study, a significant association of sex ($p<0.05$), physiologic condition ($p<0.01$) and reproductive disorders ($p<0.01$) with seroprevalence of brucellosis was observed which is in agree with those findings of Nahar and Ahmed (2009). Seropositivity in the bucks showed cross transmission of infection among sexes. On sex and breed distribution, brucellosis is known to be neither breed nor sex specific (Ajogi *et al*., 2002).

Table 1. Factors signifcantly associated with brucellosis in goats

Factors	p-value	Odds ratio	95% Confidence Interval (CI)
Sex	0.002	23	3.08, 173.62
Abortion	0.05	2.1	1.21, 4.53
Retained placenta	0.001	47.1	5.3, 416.6
Physiologic condition (Pregnancy)	0.004	6.8	1.13, 5.32

Table 2. Distribution of brucellosis detected by Milk Ring Test (MRT) in goats

S/N	Factors	No. of milk sample tested	No. of milk positive by MRT	Prevalence (%)
1	**Age (years)**			
	0-4	217	27	12.44
	>4	25	6	24.00
	Sub total	242	33	13.64
2.	**Lactation stage**			
	Early (<3 months)	153	18	11.76
	Mid (3-4months)	50	8	16.00
	Late (>4 months)	39	7	17.94**
	Sub total	242	33	13.64
3	**Rearing system**			
	Farming goat	216	30	13.89
	Rural goat	26	3	11.53
	Sub total	242	33	13.64
4	**Physiologic condition**			
	Pregnant	78	18	23.08**
	Non pregnant	164	15	9.14
	Sub total	242	33	13.64
5	**History of abortion**			
	Abortion	58	15	25.86**
	No abortion	184	18	9.78
	Sub total	242	33	13.64
6	**History of retained placenta**			
	Present	42	12	28.57*
	Absent	200	21	10.50
	Sub total	242	33	13.64

**Significant at 1% Level ($p<0.01$) and *Significant at 5% Level ($p<0.05$)

Table 3. Distribution of brucellosis detected by serological test (RBT & MAT) in goats

S/N	Factors	No of sera tested	No & % of sera positive by RBT	No & % of sera positive by MAT
1	**Age (Years)**			
	0-4	183	6(3.28)	5(2.73)
	>4	25	2(8.00)	2(8.00)
	Sub total	208	8(3.85)	7(3.37)
2	**Sex**			
	Buck	30	1(3.33)	1(3.33)
	Doe	178	7 (3.93)	6(3.37)**
	Sub total	208	8(3.85)	7(3.37)
3	**Rearing system**			
	Farming goat	184	7(3.80)	6(3.26)
	Rural goat	24	1(4.11)	1(4.11)
	Sub total	208	8(3.85)	7(3.37)
4	**Area of sample collection**			
	BAU veterinary clinic	24	1(4.17)	0(0.00)
	Babul's farm	8	0(0.00)	0(0.00)
	Savar goat farm	61	2(3.28)	2(3.28)
	Rajshahi goat farm	115	5(4.35)	5(4.35)
	Sub total	208	8(3.85)	7(3.37)

Table 3. Contd.

S/N	Factors	No of sera tested	No & % of sera positive by RBT	No & % of sera positive by MAT
5	**Physiologic condition (Doe)**			
	Pregnant	48	5(10.41)	4(8.33)*
	Non-pregnant	130	2(1.53)	2(1.53)
	Sub total	178	7(3.93)	6(3.37)
6	**History of abortion (Doe)**			
	Abortion	22	6(27.27)	5(22.72)*
	No abortion	156	2(1.28)	2(1.28)
	Sub total	178	7(3.93)	6(3.37)
7	**Reproductive disorder (Doe)**			
	Retained placenta	20	6(30.00)	5(25.00)*
	No retained placenta	158	2(1.27)	1(1.27)
	Sub total	178	7(3.93)	6(3.37)

* Significant at 5% Level ($p<0.05$) and ** Significant at 1% Level ($p<0.01$)

Brucellosis is a disease of both public health and economic importance. To have a better understanding of brucellosis in goat, a large scale survey is needed. But before doing that the tests like MRT, RBT, MAT or ELISA should be validated first for choosing one or more best performed tests.

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