PREVALENCE OF BRUCELLOSIS IN DAIRY CATTLE IN ORGANIZED AND SMALLHOLDER FARMS IN SOME SELECTED AREAS OF BANGLADESH

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ABSTRACT

Brucellosis causes a great economic loss to the livestock industries through abortion, infertility, birth of weak and dead off spring, increased calving interval and reduction of milk yield and it is endemic in Bangladesh. In this study we collected milk and blood samples simultaneously from 533 cows of Central Cattle Breeding and Dairy Farm, Savar, Dhaka and different Upazilas of Gaibandha and Mymensingh District. Five hundred thirty three samples were examined for antibodies to Brucella using the Milk Ring Test (MRT) and Rose Bengal Test (RBT). Overall 2.62 % of milk samples were positive according to MRT, while 2.06 % of the serum samples were positive to the RBT. Only 6 (1.13 %) of the samples were positive for both test (MRT and RBT). Out of 312 samples only 10 (3.20 %) were positive to MRT while 8 (2.06%) were positive to RBT in Holstein Friesian cross (p>0.05) on the other hand out of 221 samples only 4 (1.80%) were positive to MRT while 3 (1.35%) were positive to RBT in Sahiwal cross .The prevalence of brucellosis was significantly higher in the age group of > 5 years than other age groups (p≤0.01) on both test (MRT 2.75%and RBT 2.25%).Based on parity, significantly higher prevalence (MRT 2.93% and RBT 2.44%) of MRT and RBT were obtained in parity 3-5 in comparison to other parity group (p≤0.01). It is, however, obvious that although the MRT is 1st-line screening tests for brucellosis in cows in some countries, their lack of specificity is of concern. Therefore, the requirement for other confirmatory tests that are more specific should be used for the diagnosis of the disease, especially in Bangladesh.

Key words: Brucellosis, Cows, Breed, Age, Parity, Zoonosis, MRT, RBT, Diagnosis, Serology

INTRODUCTION

Brucellosis is a disease of domestic and wild animals that can be transmitted to human. Brucellosis is a recognized public health problem with worldwide distribution and one of the major causes of morbidity. The *Brucella* species especially *Brucella melitensis* and *Brucella suis* are potential agents of biological terrorism (Meslin, 1997 and Wright, 2001). A high prevalence in certain geographic areas is well recognized, although largely underestimated. Brucellosis causes a great economic loss to the livestock industries through abortion, infertility, birth of weak dead offspring, increased calving interval and reduction of milk yield and it is endemic in Bangladesh (Roth *et al.*, 2003). Brucellosis is a major zoonosis caused by small non motile gram negative and intracellular Cocco bacilli belonging to the genus *Brucella*. *Brucella* spp are facultative intracellular bacteria causing chronic disease which may persist for the whole of the affected organism. In animals brucellosis affects reproduction, fertility and reduces newborns survival and also milk productions. The mortality in adult animals is insignificant (Sewel and Blocklesby, 1990). In human the symptoms of brucellosis are weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, night sweats and chills marked asthenia and anorexia (Huge-Jones, 2000). Brucellosis is endemic in Bangladesh (Amin *et al.*, 2005; Rahman *et al.*, 2006; Uddin and Rahman, 2007; Nahar and Ahmed, 2009; Rahman *et al.*, 2009, 2010, 2011; Ahasan *et al.*, 2010). In order to control and eradicate brucellosis from human and livestock, it is very essential to establish an appropriate serological method for diagnosis of brucellosis in endemic areas. The classical serological test like MRT and RBT are known to produce cross reaction with other gram negative bacteria having antigenic similarities with brucella (Kittelrberger *et al.*, 1988) and therefore produce a lot of false positive reaction. Every year a lot of undiagnosed cases of abortion, stillbirth and retained placenta are reported in cattle of Bangladesh which might be caused by *Brucella*.

Despite preventive and control measures that exist in developing state, there is still a high potential for transmission and spread of *Brucella* spp.via animals and their products and bye-products imported from these countries (Acha *et al.*, 1987). Prevalence of brucellosis in cattle might constitute a significant hurdle for the

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development of livestock in Bangladesh. So early and accurate diagnosis is important for under taking an effective control measure against brucellosis. The MRT and RBT are widely used for screening of brucellosis exclusively in eradication programs (Sarker *et al.*, 2014). The RBT is a simple agglutination technique. Because this test does not need special laboratory facilities and is simple and easy to perform, it is used to screen sera for antibodies to Brucella (Cadmas *et al.*, 2008). These tests were chosen because they are less cumbersome to perform on a large scale and/or require no special equipment and expertise compared with other commonly used assays such as CFT and ELISA. Therefore the present study was undertaken to know the prevalence of brucellosis in cattle in organized and smallholder dairy farms in some selected areas of Bangladesh.

MATERIALS AND METHODS

As serological samples, venous blood samples were collected from jugular vein aseptically from sexually matured cattle of female sexes. A total of 533 blood samples were collected from cows of Central Cattle Breeding and Dairy Farm, Savar, Dhaka and all upazilas of Mymensingh and Gaibandha district of Bangladesh (Table 1). Milk samples were also collected from the same cows. At the time of sampling, questionnaire based data on age, sex, Breeds, location, calving status, disease history, parity were recorded. The Milk Ring Test (MRT) and Rose Bengal Test (RBT) were performed with the milk and blood samples respectively as screening tests for brucellosis. *Brucella abortus* strain 1119-3 (Dae Sung Microbiological lab, South Korea) were used for RBT.

Rose Bengal Test (RBT)

The RBT was performed according to the procedure as described by Uddin *et al*. (2007). The test serum samples *Brucella abortus* antigen (William James House, Cowley Rd. Cambridge, CB4 OWX, UK) were kept 1 hour in room temperature before beginning the test. 30 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 shaked 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 80-100 rpm for 4 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 (Fig. 1).
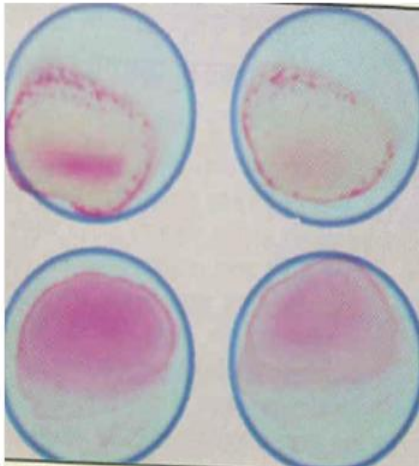

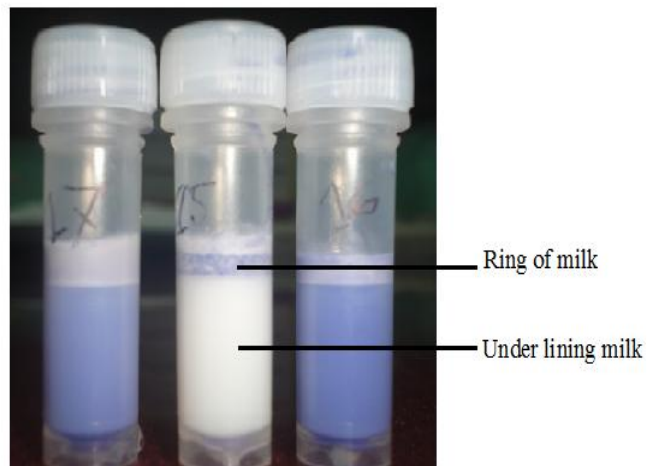Fig. 1. Rose Bengal Test (RBT). Upper row positive and lower row.

Fig. 2. Milk Ring Test. Milk in the middle tube indicates positive result showing ring of cream more colored than underlying milk. Milk in the two corner tubes indicate negative result shows ring of cream less colored than underlining milk.

Milk Ring Test (MRT)
Milk Ring test was performed by following the manufacturer's instruction (National Veterinary Services Laborotories, USDA, Ames, Iowa). In brief, antigen was kept at room temperature (18-23°c) for 1 hour before starting the test. After proper mixing, 1.0 ml of milk sample and 50 µl of MRT of antigen was 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 to 20 hours. The result was read as positive if the ring of cream equally or more colored than the underling milk and as negative if the ring of cream less colored than the underlying milk (Fig. 2).

Statistical analysis
The Chi-square test (x^2) was performed to find out the relationship between the prevalence of brucellosis and demographic variables of cows.

RESULTS
Blood samples and milk samples were collected from 533 cows from Central Cattle breeding and Dairy Farm, Savar, Dhaka and all upazilas of Gaibandha and Mymensingh Districts of Bangladesh. Out of 533 samples 14 milk samples (2.62 %) and 11 serum samples (2.06 %) were positive milk and serum screened according to MRT and RBT respectively (Table 2). Six samples (1.13 %) were positive in both the tests like MRT and RBT. The highest positive rate of 3.20% was obtained among the Holstein Friesian cross of cows using MRT, while the highest positive rate of 2.56% obtained among the Holstein Friesian cows using RBT (Table 2). Age-wise prevalence of brucellosis based on MRT and RBT were 2.26 % and 1.50 % respectively in 1-4 years age group. On the other hand prevalence of brucellosis based on MRT and RBT were significantly higher (2.75% and 2.25%) in > 5 years age group (p≤ 0.01) than those of other age group (Table 3). Based on parity, significantly higher prevalence 2.93% and 2.44% of MRT and RBT were obtained respectively in parity 3-5 in comparison to other parity group (p≤0.01) (Table 3).

Table 1. Milk and sera samples collected from CCB & DF Gaibandha and Mymensingh District

	Study area	No of sample collected	
		Blood Sample	**Milk sample**
CCB & DF		120	120
	Mymensingh Sadar	82	82
	Muktagacha	21	21
	Phulbaria	23	23
	Trishal	13	13
	Valuka	15	15
	Guforgoua	11	11
Mymensingh District	Isorgonj	18	18
	Gouripur	22	22
	Nandaiyl	12	12
	Haluaghat	30	30
	Dhobaura	25	25
	Taracanda	26	26
	Phulpur	22	22
	Gaibandha Sadar	10	10
	Palashbari	24	24
	Sundarganj	12	12
Gaibandha District	Gobindogonj	12	12
	Shaghata	11	11
	Sadullapur	14	14
	Phulceri	10	10
Total		**533**	**533**

CCB & D F- Central Cattle Breeding and Dairy Farm, Savar, Dhaka

Table 2. Breed wise prevalence of brucellosis based on MRT and RBT in cattle

Breeds of cattle	No of cows tested	MRT positive	Prevalence on MRT %	RBT positive	Prevalence on RBT %	Level of significance
Holstein Friesian cross	312	10	3.20	8	2.56	
Sahiwal cross	221	4	1.80	3	1.35	NS
Total	533	14	2.62	11	2.06	

NS=Non-significant (p>0.05)

Table 3. Age and parity-wise prevalence of brucellosis in cattle based on MRT and RBT

Parameter		No of cows tested	MRT positive	Prevalence on MRT %	RBT positive	Prevalence on RBT %	Level of significance
Age	1-4 years	133	3	2.26	2	1.50	**
	>5 years	400	11	2.75	9	2.25	
Parity	1-2	123	2	1.63	1	0.81	**
	3-5	410	12	2.93	10	2.44	

** Significant at p≤0.01

DISCUSSION

The overall prevalence of brucellosis based on MRT and RBT was recorded as 2.62% and 2.06%, respectively. The MRT is generally used for screening; other tests are required for confirmatory diagnosis. This is an agreement with Morgan (1967) who stated that the test should be used in conjunction with the established tests and not instead of them. The higher prevalence by MRT might result from false positive which could be due to many causes including mastitis, colostrums, collection at the end of lactation period or a hormonal disorder (Bercovich and Moerman, 1979). Carefully samples were collected from dairy cows excluding mastitis infected and recently delivered cows to avoid false positive reactions. It has been shown that different serological tests used for the diagnosis of brucellosis vary considerably in their ability to detect anibodies of a particular immunoglobulin class. Infected animals may or may not produce all antibody isotypes in detectable quantities (FAO, 2004). Vaccination against brucellosis is not carried out in Bangladesh, so that our result may reflect natural infection. In general the MRT have been shown in other studies to have high sensitivity but lower specificity. The MRT is not normally used on individual animals because of false positives (less specifity).

Based on outcome of the study, it is suggested that although MRT and RBT are generally useful for screening for brucellosis especially in developing countries where other tests are cumbersome to perform on a large scale and require special equipment and expertise these tests still have limitations where vaccination or medical records are not available. As a result of these limitations other confirmatory tests like ELISA, CFT, SAT shall be carried out in conjunction with MRT and RBT due to confirm the brucellosis status of cattle in Central Cattle Breeding and Dairy Farm, Savar, Dhaka and Different Upazilas of Gaibandha and Mymensingh District. The ELISA is an available assay for use on milk and serum and is very useful where large number of samples require testing. Milk ELISA is used on pooled samples which is more cost effective than testing individual animals. Although these tests may be very expensive they are needed to confirm the brucellosis status of cattle in our study area in order to safeguard the general public and in particularly that of the people directly involved in the meat inspection, milk collectors and meat milk processing. The older animal supposed to be more infected because of more contact with infectious agents and sometimes become more susceptible from malnutrition during pregnancy. It appears that the prevalence of infection in animals more than 5 years of age compared to younger animals and higher prevalence of brucellosis among older cows might be related to maturity with the advancing age. Seropositivity to be considered due to natural infection occurred because vaccination in cows has never been practiced in Bangladesh. The prevalence and severity of disease may vary with the breed, geographic location, types of diagnostic tests, husbandry and environmental factor as well as the biovar of the organism.

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