

< Short Communication >

Seroprevalence of brucellosis in cattle in selected area of Bangladesh and comparison between Rose Bengal test and i-ELISA used for the screening of brucellosis

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Abstract

Brucellosis, a bacterial zoonoses caused by the genus *Brucella* is responsible for abortion and infertility in cow. Brucellosis is causing economic loss in dairy industries and prevalent worldwide including Bangladesh but limited studies are devoted to determine the prevalence and its association with reproductive factors of dairy cows in Bangladesh. Therefore, the present study was conducted to determine the seroprevalence of brucellosis in dairy cattle using screening test Rose Bengal test (RBT) and the positive sera were further confirmed by indirect-ELISA. For this purpose, a total of 400 serum samples from dairy cows with history of abortion and various reproductive disorders were collected from the Kurigram district of Bangladesh for the detection of *Brucella* antibody. The overall prevalence of brucellosis in dairy cattle was 2.25%. Brucellosis in cases of abortion and repeat breeding was 8.3% and 2.8%, respectively. The results shows higher prevalence of brucellosis in cases of abortion followed by repeat breeding, while there was no seropositive cases from other reproductive disorders. Age-wise seroprevalence was found 3.0% in 2~3 years age group and 2.0% in 4~8 years age group. The prevalence of brucellosis in indigenous and cross-bred cattle was 3.6% and 1.7%, respectively. All the animals detected positive to brucellosis by RBT were not found to be positive by i-ELISA. However, the RBT might be a suitable screening test for the diagnosis of *Brucella* infection in field condition in Bangladesh. These data will help to develop effective disease prevention strategies.

Key words : Brucellosis, Cattle, Rose Bengal test, i-ELISA, Bangladesh

INTRODUCTION

Brucellosis is a zoonotic disease caused by gram-negative bacteria *Brucella* that are pathogenic for a wide variety of animals and human beings. The main domestic animals affected are cattle, sheep, goats, and pigs (Young, 1995). While the genus *Brucella* has six species on the basis of host specificity, *B. abortus* caused

abortion and infertility in dairy cows. It also causes significant reproductive disorders in sexually mature cattle manifested by late term abortions, inflammation of the genital organs and fetal membrane, fetal death, sterility, and formation of localized lesions in the lymphatic system and joints (Probert et al, 2004). Brucellosis is an occupational hazard that affects farmers and their families, abattoir workers, farm labor, slaughter-house workers, butchers and veterinarians (Yagupsky and Baron, 2005).

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Brucellosis is endemic worldwide including Bangladesh (Amin et al, 2004; Das et al, 2008). But often a neglected disease. In Bangladesh it was first reported in cattle (Mia and Islam, 1967), and in human (Rahman et al, 1983). Islam et al (1983) estimated the annual economic loss in Bangladesh due to bovine brucellosis in indigenous cows as 720,000 EUR (total) and 12,000 EUR per 1,000 cross-bred cows and a total of 276,000,000 EUR in cross-bred cows. There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which are thought to be due to brucellosis and these have a significant impact on the development of livestock in Bangladesh (Islam et al, 1983; Rahman et al, 2006; Rahman, 2010).

However, in spite of its importance, few studies are devoted to determine the prevalence of brucellosis in dairy cattle in Bangladesh. Therefore, it is necessary to determine the prevalence of brucellosis and its association with different reproductive disorders in dairy cattle.

The diagnosis of brucellosis is confirmed by isolation of *Brucella* spp. by bacteriological culture or by the detection of an immune response by serological test to its antigens (Orduña et al, 2000). But the diagnosis of brucellosis based exclusively on *Brucella* isolation presents several drawbacks. The slow growth of *Brucella* may delay diagnosis for more than 7 days and also, the sensitivity is often low, ranging from 50 to 90% depending on disease stage, *Brucella* spp., culture medium, quantity of bacteria and culture technique employed (Gotuzzo et al, 1986). Hence, the serological tests are important for diagnosis of brucellosis. Serological test like the Rose Bengal test (RBT), slow agglutination test (SAT), mercaptoethanol test and enzyme linked immunosorbent assay (ELISA) and complement fixation test (Islam, 1983; Ferreira et al, 2003) are generally used for the detection of *Brucella* infection in animal. RBT has been widely used and more useful as a screening herd test (OIE, 2009). However, it is necessary to find out an appropriate test that could be performed easily with less expense but has high sensitivity in field condition. To the best of knowledge, there is no such study conducted in field condition in Bangladesh. Therefore, the aim of this study was to determine the prevalence of the brucellosis in dairy cattle in selected regions (six upazilas

of Kurigram district) in Bangladesh by detecting the *Brucella* antigen by RBT and i-ELISA.

MATERIALS AND METHODS

A total of 400 blood samples were collected from dairy cattle aged over ≥ 2 years with the history of repeat breeding, anoestrus, retention of placenta, dystocia, prolapse of uterus and abortion of six upazilas in Kurigram district. Total cattle population in Kurigram district is 0.71 million and there are 457 registered dairy farms in Kurigram district. There is a corridor in Kurigram Sadar for importation of cattle from India (DLO Office Kurigram, 2007). There are lots of cases for abortion, repeat breeding, retention of placenta and other reproductive disorders are frequently reported in different regions in Kurigram district which are thought to be for brucellosis. So far literature reviewed, there was no survey carried out in Kurigram district for the determination of prevalence of brucellosis in dairy cattle. Therefore, six upazilas of Kurigram district were considered for this study. Number of serum samples collected from Ulipur, Rajarhat, Kurigram Sadar, Nageswari, Roumari, and Chilmari upazilas were 100, 120, 90, 50, 20, and 20, respectively. The study was conducted for a period of 6 months from June, 2011 through November, 2011 in the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Rose Bengal Test (RBT)

All the blood samples were processed for serum preparation and then subjected to RBT as a screening test using *B. abortus* antigen (obtained from Dae Sung Microbiological lab, South Korea). The test was performed according to the procedure as described by the OIE (2009).

Indirect enzyme linked immunosorbent assay (i-ELISA)

All the samples found to be positive in RBT were

further confirmed using i-ELISA in the OIE reference laboratory for brucellosis in Jena, Germany. The assay was performed according to the protocol provided by the manufacturer's instructions (Svanova Biotech AB, Uppsala, Sweden).

Statistical analysis

The Chi-square test was employed to find out the significant differences in the prevalence in terms of age, breed and different reproductive disorders of cattle.

RESULTS

Seroprevalence of brucellosis

The overall prevalence of brucellosis was 2.25% (9/400) in dairy cattle of six upazilas of Kurigram district. It is to be mentioned that the doubtful negative samples (n=22) that were tested in the OIE reference laboratory for brucellosis in Jena, Germany gave also negative reaction. The highest prevalence (5.6%) was recorded in Kurigram Sadar upazila while 2.0% and 1.7% were recorded in Ulipur and Rajarhat upazilas, respectively. However, there was no significant difference in the prevalence of these three upazilas. No positive reactors were found in three other upazilas. Results of age-wise seroprevalence of brucellosis revealed that the prevalence was 3.0% in 2~3 years age group and 2.0% in 4~8 years age group. With respect to various reproductive problems, the number of positive reactors was recorded and it was found that there were no positive reactors with the history of anoestrus, dystocia, prolapse of uterus and retention of placenta. However 2.8% and 8.3% prevalence was recorded in dairy cattle with history of repeat breeding and abortion. Breed-wise seroprevalence recorded in the study revealed that the prevalence of brucellosis in indigenous and cross-bred dairy cattle was 3.6% and 1.7% respectively and there was no significant difference in the prevalence between the types of breed (Table 1).

Notably, the nine RBT positive samples including 22 doubtful samples were tested by using i-ELISA. The re-

Table 1. Seroprevalence of brucellosis in Kurigram district based on different demographic factors

Upazilas	No. of serum samples tested	No. of RBT positive reactors	Prevalence (%)
Ulipur	100	2	2.0*
Rajarhat	120	2	1.7*
Kurigram Sadar	90	5	5.6*
Nageswari	50	0	-
Rowmari	20	0	-
Chilmari	20	0	-
Age			
2~3 years	100	3	3.0*
4~8 years	300	6	2.0*
Reproductive problems			
Repeat breeding	250	7	2.8*
Abortion	24	2	8.3*
Anoestrus	64	0	0
Dystocia	32	0	0
Prolapse of uterus	8	0	0
Retention of placenta	22	0	0
Breed			
Indigenous	111	4	3.6*
Cross	289	5	1.7*

*Values with same superscripts within a column do not differ significantly at 5% level of significance.

sults showed that all the doubtful samples were negative in the case of i-ELISA. Additionally, three samples out of the nine samples detected positive by RBT were negative in the case of i-ELISA.

DISCUSSION

Various studies have been conducted in Bangladesh to determine the prevalence of the disease in bovine species (Rahman et al, 2006; Nahar and Ahmed, 2009; Kadir, 2010) but the present study is based mainly on non-random sampling techniques from dairy cattle with reproductive problems. Only animals aged over ≥ 2 years were tested since brucellosis is a disease of sexually mature animals. The RBT is considered as the most widely used test for the screening and monitoring of brucellosis in dairy cattle (OIE, 2009). In the last decade, several indirect enzyme linked immunosorbent assays (ELISA) have been developed and successfully tested on sera samples.

The present study was designed to determine the se-

reprevalence of the disease in the dairy cattle of Kuri-gram district using RBT and to compare the RBT with i-ELISA. The serum samples from selected dairy cattle with history of reproductive problems were analyzed with RBT which revealed prevalence of 2.25%. This finding is in agreement with the reports of some research (Amin et al, 2004; Kadir, 2010; Rahman et al, 2006) who reported the prevalence 2%, 2.4%, 2.66% and 2.72%, respectively. The result is lower than the findings of Nahar and Ahmed (2009) who reported 4.5%. This variation in the prevalence may be due to variation in the age, breed, sex, pregnancy status of the animal, study area, hygienic condition, breeding techniques, herd size, reproductive diseases and diagnostic tests applied (Kebede, 2008). The prevalence in Kuri-gram Sadar upazila is higher (5.6%) than Ulipur (2.0%) and Rajarhat (1.7%) upazilas. The difference in the prevalence among the upazilas was not statistically significant.

Age-wise disease prevalence was found 3.0% in 2~3 years age group while 2.0% in 4~8 years age group. The difference between the groups was not statistically significant. In contrast to the findings of the present study, Rahman et al (2011) reported the prevalence of brucellosis in the cow aged 2.5~4 years as 1.45 % while in the cows over four years of age as 5.31%. Similarly, Amin et al (2004) reported 2.3% and 4% prevalence in the <4 and >4 years age group, respectively. Age-wise prevalence has also been studied by Abubakar et al (2010) who showed that the incidence of brucellosis increased with age, and the incidence is high in sexually mature animals. The prevalence of brucellosis was 2.8% and 8.3% in dairy cattle with history of repeat breeding and abortion. The present findings are in agreement with Rahman et al (2006) who reported brucellosis to be higher in cattle with a history of abortion (15%) as compared to those with a history of returns to service (1.45%). In this study, the prevalence of brucellosis was 3.6% and 1.7% in the indigenous and cross-bred cattle, respectively. The difference between the two groups was not statistically significant. Akbarmehr and Ghiyamirad (2011) reported that there exists differences in the prevalence of brucellosis in different breeds, but statistically not significant.

In the present study, i-ELISA in conjunction with RBT was employed to compare the efficacy, testing of 31 samples by both RBT and i-ELISA revealed that 3 samples were positive and 22 were negative by both the tests while additional six samples were negative by i-ELISA only. Chakraborty et al (2000) reported lower sensitivity (56.96%) and higher specificity (96.77%) of RBT, whereas Singh et al (2004) found 88.46% sensitivity and 97.75% specificity. On the other hand, Ahmed et al (2011) reported higher sensitivity (100%) and specificity (94.92%) of RBT. Additionally, higher sensitivity and lower specificity of RBT were also obtained by Muma et al (2007).

In spite of the facts, we could conclude that RBT could be an important screening test for brucellosis in Bangladesh and the doubtful and positive cases could be further validated or confirmed using i-ELISA. This study showed that prevalence of brucellosis is related to the reproductive problems of cows in Bangladesh. These results will help the livestock department to design preventive measure for brucellosis which is causing huge economic losses in Bangladesh.

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