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**<Original Article>**

# Serological prevalence of brucellosis of cattle in selected dairy farms in Bangladesh

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**Abstract :** This study was conducted to investigate the status of brucellosis in dairy cattle from five selected dairy farms in the Mohammadpur Beribadh area of Bangladesh. A cross-sectional study was carried out from October 2010 to March 2011 in which a total of 334 serum samples from cattle in five herds were screened by the Rose-Bengal plate-agglutination test (RBPT) and the positives were confirmed using an indirect enzyme-linked immunosorbent assay (I-ELISA). A structured questionnaire was used to collect epidemiological information describing the animals. Overall, 4.20% of the animals were RBPT positive, while subsequent confirmatory tests with I-ELISA revealed that the overall animal-level prevalence derived from the samples was 1.20%. Additionally, the prevalence was relatively higher in females than in males. A significant association was found between abortion, age of the animals, and the occurrence of brucellosis ($p < 0.05$). Considering the overall low prevalence of brucellosis in the selected farms in the present study, a brucellosis eradication program for dairy farms using a test-and-slaughter policy would be possible.

**Keywords :** Bangladesh, brucellosis, indirect enzyme-linked immunosorbent assay, Rose-Bengal plate-agglutination test, seroprevalence

## Introduction

Brucellosis is one of the most important and widespread zoonoses in the world. The disease caused by different species of the genus *Brucella*, a Gram negative, non motile, non spore forming, and rod shaped bacteria [4]. *Brucella abortus* infection in cattle causes a huge economic losses by decreased calving percentage, delayed calving, culling for infertility, cost of treatment, decreased milk production, abortions, stillbirth, birth of weak calves and loss of man-hours in infected people [18]. Infection in bulls also causes orchitis, epididymitis, seminal vesiculitis and hygroma [8, 11].

In the agro-based economy of Bangladesh, livestock contribute 2.73% of the total GDP and 80% of rural people are directly or indirectly involved with livestock rearing. There are an estimated 23.4 million cattle, 1.86 million buffaloes, 33.5 million goats, 1.1 million sheep being reared in Bangladesh. The importance of brucellosis has not been precisely known, but it can have a considerable impact on human and animal health, as well as on socioeconomic factors, as rural income relies largely on livestock breeding and dairy products and people usually live in very close proximity with their livestock. There are 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 [9, 20, 23]. In Bangladesh, brucellosis was first identified in cattle in 1967 [14], in buffalo in 1997 [19] and others reported brucellosis in one or two species of livestock as well as humans [2, 3, 15, 17, 21]. Human brucellosis is caused by exposure to livestock and livestock products. Infections can result from direct contact with infected animals and can be transmitted to consumers through raw milk and milk products. Prevalence of brucellosis has been reported in cattle from different parts of the world. The prevalence of brucellosis in cows of better managed farms has also occurred and estimated of human brucellosis as 12.8% in herders and agricultural workers and 21.6% in goat farmers [16]. The seroprevalence of brucellosis in cattle was 2.4%~18.4% whiles the herd-level seroprevalence in cattle was 62.5% in Bangladesh [20].

Serological testing using the Rose-Bengal plate-agglutination test (RBPT), serum agglutination test, tube agglutination test, mercaptoetanol test and enzyme-linked immunosorbent assay (ELISA) are generally used for the detection of *Bru-*

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*cella* infection in livestock. Recently a simple and rapid field test for the 'penside' diagnosis of brucellosis in livestock has been developed [1]. ELISA has been evaluated as a serological test for many years for its diagnostic performance to detect serum antibody for brucellosis in domestic animals. It has gained popularity over recent years as an alternative to other serological tests. ELISA for diagnosis of brucellosis has several advantages when compared with other tests; Firstly, ELISA is a direct method of Identification of specific antibody and therefore, it is not prone to false positive reactions. Secondly, it is more sensitive than other the agglutination test and thus has the potential to detect infected animals. Thirdly, the antibody enzyme conjugate employed has light chain reactivity and thus is able to detect all classes of antibody. A combine determination of all classes of antibody allows accurate serological diagnosis at any stages of disease. Fourthly, ELISA results provide an epidemiological tool for investigation the infective status of flocks in places where vaccination has never been practiced, like Bangladesh [22]. This is an important study with regard to public health, and it may help to control this zoonotic disease efficiently in the capital city of Bangladesh.

## Materials and Methods

### Study area

The Mohammadpur Beribadh is located in the north-west corner of Dhaka district and lies between latitude 23°46' N and longitude 90°23' E. The study was carried out in the cross-bred dairy cattle farms of Mohammadpur Beribadh area between October 2010 and March 2011. Confirmatory diagnosis was performed in Bangladesh Livestock Research Institute (BLRI) laboratory, Savar, Dhaka.

### Study period and experimental design

A cross-sectional study design was used to find out the prevalence of brucellosis in cattle, particularly in cross-bred dairy cattle, in Mohammadpur Beribadh, Dhaka. Samples were collected, and questionnaires have been administered to each farm from October 2010 to March 2011.

As serological samples, venous blood samples were randomly and aseptically obtained from cross-bred cattle of both sexes. Blood was collected from the jugular veins into Vacutainer tubes (Becton, Dickinson and Company, USA), which were immediately placed into an ice bath and transported to the laboratory within a maximum of 7 h. The clot was allowed to form in the Vacutainer tube in the field before transportation. The samples were centrifuged at 1,500 × g for 15 min and the serum was removed and stored at −20°C until analyzed.

A total of 334 blood sera samples were collected from the dairy cattle farms of Mohammadpur Beribadh area. Among cattle sera samples, 106 from Zaker dairy farm, 87 from MS dairy farm, 66 from Haque dairy farm, 43 Jamal dairy farm and 32 from Dhaka dairy farm. The study also recorded required clinical, epidemiological and reproductive information. During sampling a questionnaire based on age, sex, area, pregnancy status, disease history, reproductive problems such as abnormal uterine discharge, abortion and reproductive diseases was filled out. All blood samples were processed for sera preparation and were tested with Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) for confirmatory diagnosis. The serum sample was tested using RBPT, and if this was positive, the positive sample was re-tested with I-ELISA for confirmation. If either of these second tests were positive, the cow was classified as infected and the animal was designated as 'reactor' and considered infected with brucellosis.

### RBPT and I-ELISA

All the blood samples were processed for sera preparation and then subjected to RBPT as a screening test in order to identify animals infected with brucellosis and the results were confirmed by I-ELISA (Svanova Biotech AB, Sweden). RBPT was performed according to the procedure described by the World Organization for Animal Health (OIE) [25]. The test serum samples and Rose-Bengal antigen were kept for 1 h at room temperature before the beginning of the test. The test result was considered as positive when there was any degree of agglutination noticeable, and the absence of agglutination was considered as negative. I-ELISA was performed according to the protocol provided by the ELISA kit manufacturer company. All optical density (OD) values for the test samples as well as the negative control (Neg C) are related to the OD value of the positive control as the following formula:

$$\text{PPV (percent positivity value)} = \frac{\text{Test sample or Neg C (OD)}}{\text{Positive control (OD)}} \times 100$$

### Statistical analysis

The questionnaire-based data was processed using Microsoft Excel 2003 to calculate the descriptive statistics. Database errors were also rechecked by using the Microsoft Excel program. The results were statistically analyzed and make interpretation using Chi-square tests ($\chi^2$). All tests of statistical significance were carried out at the $p < 0.05$ level unless otherwise stated.

## Results

### Overall seroprevalence of brucellosis in dairy cattle in the selected farms

A total 334 serum samples were collected from the selected dairy cattle farms of Mohammadpur Beribadh, Savar, Dhaka, Bangladesh. The overall animal-level seroprevalence of brucellosis within the herd was 1.20% (4/334) confirmed by I-ELISA. The number of positive reactors by RBPT was four of 106 (3.80%) in Zaker dairy farm, five of 87 (5.70%) in

**Table 1.** Overall herd-level seroprevalence of brucellosis in dairy cattle of the selected farms based on RBPT and I-ELISA

| Herd | n | RBPT positivity (%) | I-ELISA positivity (%) |
|---|---|---|---|
| Zaker dairy | 106 | 4 (3.80) | 1 (0.94) |
| MS dairy | 87 | 5 (5.70) | 2 (2.30) |
| Haque dairy | 66 | 3 (4.50) | 1 (1.50) |
| Jamal dairy | 43 | 1 (2.30) | 0 (0.00) |
| Dhaka dairy | 32 | 1 (3.10) | 0 (0.00) |
| Overall | 334 | 14 (4.20) | 4 (1.20) |

n, sample size; RBPT, Rose Bengal plate-agglutination test; I-ELISA, indirect enzyme-linked immunosorbent assay.

**Table 2.** Animal-level seroprevalence in cattle in five farms by age, sex, pregnancy status and history of abortion based on RBPT and I-ELISA

| Variable and level | n | Number of positive reactors by RBT (%) | Number of positive reactors by I-ELISA (%) |
|---|---|---|---|
| All animals | | | |
| Age | | | |
| < 2 years | 17 | 1 (5.88) | 0 (0.00) |
| 2~4 years | 274 | 10 (3.64) | 3 (1.09) |
| 4 > years | 43 | 3 (6.97) | 1 (2.32)* |
| Sex | | | |
| Male | 80 | 3 (3.75) | 0 (0.00) |
| Female | 254 | 11 (4.33) | 4 (1.57) |
| Females only | | | |
| Pregnancy status | | | |
| Pregnant | 86 | 4 (4.60) | 2 (2.32) |
| Non-pregnant | 168 | 10 (5.95) | 2 (1.19) |
| History of abortion | | | |
| Abortion | 7 | 2 (28.57) | 1 (14.57)* |
| No abortion | 247 | 12 (4.85) | 3 (1.21) |

*Significant at 5% level of probability ($p < 0.05$).

MS dairy farm, three of 66 (4.5%) in Haque dairy farm, one of 43 (2.30%) in Jamal dairy farm and one of 32 (3.10%) in Dhaka dairy farm.

The numbers of positive reactors by I-ELISA was one out of 106 (0.94%) in Zaker dairy, two out of 87 (2.30%) in MS dairy, one out of 66 (1.50%) in Haque dairy (Table 1) in the five selected dairy farms in Bangladesh. None of the samples were positive by I-ELISA in Jamal dairy and Dhaka dairy respectively, although one from each herd was positive reactor by RBPT.

**Age and sex wise seroprevalence of brucellosis in dairy cattle**

Age, sex, status of pregnancy and the history of abortion related seroprevalence of brucellosis in dairy cattle of five selected farms are shown in Table 2. The group of animals < 2 years of age showed no positive reaction but in the 2~4 years age group, the seroprevalence was 1.09% (three out of 274 samples) and in the > 4 years age group this value was 2.32% (one out of 43 samples). Regarding the sex-related seroprevalence, higher prevalence of brucellosis was found in female animals. Eighty males and two hundred fifty four females were tested and the prevalence of brucellosis was 1.57% and 0.00% in the case of females and males, respectively.

**Status of pregnancy and abortion related prevalence of brucellosis in cattle**

There was found the differences of seroprevalences between pregnant and non-pregnant cattle and was 2.32% (two out of 86 samples) and 1.19% (two out of 168), respectively. Among 254 female animals, seven were recorded a previous history of abortion. The seroprevalence of brucellosis in the aborted dairy cows was 14.57% (one out of 7 samples). Alternatively, the prevalence was relatively lower (1.21%) in those animals has no record of previous abortion. There existed a significant association in between abortion and the prevalence of brucellosis. Based on this, it can be concluded that the prevalence of brucellosis was significantly higher in animals with a previous abortion record than in animals with no abortion record.

## Discussion

Brucellosis is an important zoonosis and serological surveillance is essential to its control [7]. Although the many countries have undertaken eradication programs to control the brucellosis but economic losses from the disease by abortion and infertility can be heavy. Therefore, subsequent culling from the herds and continuous monitoring is necessary for existence of the infection. Although the eradication programs established by vaccination, test and slaughter of the infected one, but the disease still remains as a major zoonosis all over the world [4, 10, 13] and also prevalent in many countries. The numbers of brucellosis infection are still underestimated although half a million cases of brucellosis each year world is reported by the World Health Organization (WHO) [24]. In recent years, brucellosis has been eradicated from many countries from their herds, and many other countries have significantly reduced the prevalence of the infection among their livestock populations. After that, the disease is still distributed worldwide wherever rearing the livestock populations. In many underdeveloped and developing countries, the enormous economic losses are continuing in the livestock production by brucellosis and also pose a serious threat to humans [6].

The aims of the present study was to determine the seroprevalence of brucellosis in the selected dairy farms in Bangladesh and also to gather better understanding regarding the epidemiology of brucellosis in dairy cattle as well as providing disease control information. As the vaccination against brucellosis is not practiced in Bangladesh, serologically positive cases were considered as natural infection. The results obtained from this study revealed the prevalence of brucellosis in cattle in the selected farms was 1.20% by I-ELISA. The prevalence and severity of the infection may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factors [3]. Therefore, a relatively lower seroprevalence observed in this study and this may be due to the selected farms, smaller sample size and also to the applied diagnostic methods.

The present study mostly included the cross-bred dairy cattle from five selected farms which follows a semi-intensive health management system. There is also a difference in using the diagnostic methods; in the previous study where mostly RBPT was employed whereas in this study I-ELISA also used as a confirmatory test. In general, RBPT has the higher sensitivity and lower specificity compared to I-ELISA. So, when RBPT was used as a screening test, a higher prevalence can be determined. Other contributory factors to variation include contact with wild animals and housed dogs.

In the present study there was a significant relationship observed between age and the prevalence of brucellosis in cattle. According to the age-related seroprevalence in three age groups of cattle, the highest prevalence of brucellosis was found in the cattle above 4 years of age, which was 2.32% using I-ELISA. This finding is in agreement with the observation of others [5], who reported a higher prevalence of brucellosis (3.54%) in cattle aged more than 48 months. A similar finding was also reported by others [16]. In the aged cattle, the higher prevalence was recorded and this was probably due to their advanced age and for their higher survival rate of this age group to this disease, as the organism may remain latent or chronic for an unspecified period without clinical disease.

In this study, we found the higher prevalence rate of brucellosis in female cattle than males. This observations correlates with the findings of other records [12] where a higher prevalence of brucellosis among females. The higher infection rate in females might be due to infection within the reproductive tract of females, providing a potential reservoir for propagating the organism. There was also a positive association between the history of previous abortion recorded and the prevalence rate of *Brucella* infection in cattle. The prevalence of brucellosis in cattle with a previous history of abortion was recorded as 14.57%. Similarly, Ibrahim and Habiballa [8] reported a higher prevalence of brucellosis in cows that had a previous record of abortion of 14.2%. Among all of the reproductive disorders that were investigated, a history of previous abortion was associated with the highest prevalence of brucellosis was observed in this study. While the overall seroprevalence is relatively low (1.20%), brucellosis is an important threat to the health of animals and humans. Therefore, the results obtained from this study can provide valuable information regarding the epidemiology of brucellosis in farmed cattle in Bangladesh as well for controlling the infection.

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