

## Prevalence of Brucellosis in Humans and Cattle in Selected Areas of Bangladesh

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DOI: <https://doi.org/10.3329/jafmc.v14i1.42718>

### Abstract

**Introduction:** Brucellosis is a recognized public health problem and one of the major causes of morbidity both in humans and animals. It causes abortion and infertility in women and livestock as well.

**Objective:** The research was carried out to know the prevalence and to identify the risk factors of brucellosis in humans and cattle in study areas in Bangladesh.

**Materials and Methods:** The study areas were all Upazilas of Mymensingh, Jamalpur, Rangpur, Kurigram, Bagerhat and Gaibandha district and the Government owned Central Cattle Breeding and Dairy Farm (CCBDF) in Savar, Dhaka for cattle and human samples were collected from Mymensingh Medical College Hospital, Mymensingh, Bangladesh. A total livestock samples collected were 1043 and human samples were 460. Besides doing all the tests in Bangladesh Agricultural University, Mymensingh Medical College and Hospital laboratory Bangladesh, it was done also in Office International des Epizooties (OIE) world reference Laboratory in Jena, Germany.

**Results:** Out of 1043, 28 milk samples (2.68 %) and 23 serum samples (2.21 %) were positive by MRT and RBT respectively. The highest prevalence was 3.05% in the Holstein Friesian cross using MRT, while 2.49% in the Holstein Friesian cows using RBT. Age-wise prevalence of brucellosis by MRT and RBT were 2.06% and 1.23% respectively in 1-4 years age group. On the other hand prevalence of brucellosis based on MRT and RBT were significantly higher (2.88% and 2.50%) in > 5 years age group ( $p \leq 0.01$ ) than those of other age groups. Based on parity, significantly higher prevalence 3.07% and 2.58% of MRT and RBT were obtained respectively in parity 3-5 in comparison to other parity group ( $p \leq 0.01$ ). In case of human brucellosis 460 blood samples were tested with RBT, SAT, CFT, iELISA, conventional PCR and real time PCR but were negative in all tests. In case of livestock and human

brucellosis bacteria were cultured in OIE Reference Laboratory, Federal Research Institute for Animal Health, but bacteria were not isolated.

**Conclusion:** The prevalence of brucellosis is asserted in human and cattle in this study although MRT is 1st-line screening tests for brucellosis. Whereas no human samples were found positive for brucellosis in this study.

**Key-words:** Brucellosis, Human & cattle, Prevalence & risk factor.

### Introduction

Spontaneous abortions mostly in the first and 2<sup>nd</sup> trimester of pregnancy are evident in pregnant women infected with brucella<sup>1</sup>. Although endocarditis (< 2% of cases) a severe complication commonly associated with *Brucella melitensis* infection and accounts at least 80% of death due to brucellosis<sup>2,3</sup>. Lack of appropriate therapy during acute phase may result in localization of brucella in various tissues and organs lead to sub-acute or chronic disease that is very difficult to treat<sup>4</sup>. Sign-symptoms of brucellosis usually referred as pyrexia of unknown origin (PUO) can be confused with other diseases including enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors<sup>5</sup>. Symptoms of brucellosis are weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, night sweats and chills marked asthenia and anorexia<sup>6</sup>.

Brucellosis causes a great economic loss to the livestock industries through abortion, infertility, birth of weak and dead offspring, increased calving interval and reduction of milk yield and it is endemic in Bangladesh<sup>7</sup>. In animals, brucellosis affects reproduction, fertility and reduces newborns survival and also milk production. The mortality in adult animals is insignificant<sup>8</sup>. Despite preventive and control

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measures that exist in developing countries, there is still a high potential for transmission and spread of brucella via animals and their products and byproducts imported from these countries<sup>9</sup>. Prevalence of brucellosis in cattle might constitute a significant hurdle for the development of livestock in Bangladesh. So, early and accurate diagnosis is important for effective control measure against brucellosis. The milk ring test (MRT) and rose bengal test (RBT) are widely used for screening of brucellosis exclusively in eradication programs<sup>10</sup>. The RBT is a simple agglutination technique, does not need special laboratory facilities and is simple and easy to perform, it is used to screen sera for antibodies to brucella<sup>11</sup>. Human brucellosis is also known for complications and involvement of internal organs and its symptoms can be diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis and prostatitis in male<sup>12</sup>. Mothers those who are breast feeding may transmit the disease or infection to their infants and sexual transmission has also been reported<sup>13,14</sup>.

Due to the highly infectious nature of this organism, they can be readily aerosolized. Moreover, an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza<sup>15</sup>. In places where brucellosis is endemic, humans can get infected via contact with infected animals or consumption of their products and byproducts especially milk and milk products mainly cheese made from unpasteurized milk of sheep and goats and rennet from infected lambs and kids. Some specific occupational groups including farm workers, veterinarians, ranchers and meat packing employees are considered at higher risk<sup>16</sup>. Consumption of sheep and goat milk containing *Brucella melitensis* is an important source of human brucellosis worldwide and has caused several outbreaks. The prevalence of human brucellosis acquired from dairy products in some countries is seasonal, reaching a peak usually after kidding and lambing<sup>17</sup>. Therefore, the present study was undertaken to know the prevalence of brucellosis in human and cattle in some selected areas of Bangladesh.

## Materials and Methods

The study areas included all Upazilas of Mymensingh, Jamalpur, Rangpur, Kurigram, Bagerhat and Gaibandha district and the Government owned Central Cattle Breeding and Dairy Farm (CCBDF) in Savar, Dhaka, of Bangladesh and human samples from Mymensingh Medical College Hospital, Mymensingh, Bangladesh. The total livestock population was 1043 and human were 460. Carefully cattle blood samples were collected from dairy cows excluding mastitis infected and recently delivered cows to avoid false

positive reactions. This was a descriptive cross sectional study and the study areas were selected because of high cattle population and poorer area, as such peoples were found fond of rearing livestock as a source of income. CCBDF is the largest farm in Bangladesh with major objectives to produce crossbred heifers and bulls for distribution to farmers. This farm maintains a herd of about 3500 cattle round the year. All the human blood samples were collected from patient in febrile conditions and were preserved in freezing condition at -800c. All the samples were tested by RBT, MRT, serum agglutination test (SAT), compliment fixation test (CFT), indirect enzyme-linked immunosorbent assay (iELISA), conventional polymerase chain reaction (PCR) and real time PCR in Department of Medicine, Department of Microbiology & Hygiene, Bangladesh Agricultural University, Department of Microbiology, and Department Clinical Pathology of Mymensingh Medical College Hospital, Bangladesh. 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 the study area in order to safeguard the health of general public particularly those people directly involved in the meat inspection, milk collection and meat milk processing. Repetition of tests was also done in Office International des Epizooties (OIE) reference laboratory for brucellosis, Federal Research Institute for Animal Health in Jena Germany. The Chi-square test ( $\chi^2$ ) was performed to find out the relationship between the prevalence of brucellosis and demographic variables of cows.

## Results

Out of 1043 samples, 28 milk samples (2.68 %) and 23 serum samples (2.21 %) were positive for brucellosis by MRT and RBT respectively (Table 1). The highest positive rate of 3.05% was obtained among the Holstein Friesian cross of cows using MRT, while the highest positive rate of 2.49% obtained among the Holstein Friesian cows using RBT (Table 1). Age-wise prevalence of brucellosis based on MRT and RBT were 2.06% and 1.23% respectively in 1-4 years age group. On the other hand prevalence of brucellosis based on MRT and RBT were significantly higher (2.88% and 2.50%) in > 5 years age group ( $p \leq 0.01$ ) than those of other age group (Table 2). Based on parity, significantly higher prevalence 3.07% and 2.58% of MRT and RBT were obtained respectively in parity 3-5 in comparison to other parity group ( $p \leq 0.01$ )(Table 2). In case of human brucellosis among 460 samples all samples were negative to RBT, SAT, CFT, iELISA, conventional PCR and real time PCR.

**Table-I:** Breed wise prevalence of brucellosis based on MRT and RBT in cattle

Breeds of cattle	No of cows tested	MRT positive	Prevalence on MRT %	Odds ratio (95% CI)	P-value	RBT positive	Prevalence on RBT %	Odds ratio (95% CI)	P-value
Holstein Friesian cross	722	22	3.05	1.65 (0.66-4.11)	0.277	18	2.49	1.62 (0.59-4.39)	0.342
Sahiwal cross	321	6	1.87	Reference	-	5	1.56	Reference	-
<b>Total</b>	1043	28	2.68	-	-	23	2.21	-	-

**Table-II:** 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	Odds ratio (95% CI)	P-value	Prevalence on RBT %	Odds ratio (95% CI)	P-value
Age	1-4 years	243	5	2.06	3	Reference	-	1.23	Reference	-
	>5 years	800	23	2.88	20	1.41 (0.53-3.75)	0.489	2.50	2.05 (0.60-6.96)	0.239
Parity	1-2	228	3	1.32	2	Reference	-	0.88	-	-
	3-5	815	25	3.07	21	2.37 (0.71-7.93)	0.148	2.58	2.99 (0.69-12.84)	0.122

## Discussion

The overall prevalence of brucellosis based on MRT and RBT was recorded as 2.68% and 2.21% respectively. The MRT is generally used for screening; other tests are required for confirmatory diagnosis. Morgan in their study<sup>18</sup> stated that the test should be used in conjunction with the established tests and not as alternative. Higher number of positive results by MRT might develop from false positives which could be due to many causes including mastitis, colostrum, and collection at the end of lactation period or a hormonal disorder<sup>19</sup>. It has been shown that different serological tests used for the diagnosis of brucellosis vary considerably in their ability to detect antibodies of a particular immunoglobulin class<sup>20</sup>. Infected animals may or may not produce all antibody isotopes in detectable quantities<sup>21</sup>. Vaccination against brucellosis is not carried out in Bangladesh, so this study results 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 specificity). 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, but these tests still have limitations where vaccination or medical records are not available. Due to limitations other confirmatory tests like ELISA, CFT, SAT were carried out in conjunction with MRT and RBT for confirming the brucellosis status of cattle in Central Cattle

Breeding Station and Dairy Farm, Savar, Dhaka and different Upazilas of Gaibandha, Rangpur, Jamalpur and Mymensingh district. ELISA is an available assay for using on milk and serum and is very useful where large number of samples require testing. It appeared from results that the prevalence of infection is more in animals having age above 5 years comparing to younger animals and higher prevalence of brucellosis was found among older cows might be due to maturity with the advancing age. Sero-positivity what so ever is, due to natural infection 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.

In case of humans, 460 samples were found RBT negative; all were cultured in the Department of Medicine, Department of Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh and also cultured that (460 human samples) at Federal Livestock Research Institute (OIE Reference Laboratory for Brucellosis), Jena, Germany but could not identify the bacteria. In case human all samples were tested with RBT, SAT, CFT, iELISA, conventional PCR, real time PCR in OIE Reference Laboratory for Brucellosis, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institut, (FLI), Jena, Germany but were negative in all tests. In some studies; several positive cases were found positive for brucellosis in humans and goats<sup>22,23</sup> but this study examined number of samples from human, where no samples were found positive, which may be due to high hygienic and improved environmental

condition and differences in geographical location. Rahman et al<sup>24</sup> investigated that the sero-prevalence of brucellosis in humans were found 15% in dairy workers or milkers, 12.85% in cow boy and agricultural workers who experienced direct contact with animals. The individual having direct contact with animals revealed higher occurrence of the disease<sup>25</sup>. This finding is not similar to the findings of this study and the difference might be due to the time laps, variation in procedure, sanitation, hygienic management, awareness of people, treatment of human, improvements of medical services and food habit. Therefore, other confirmatory tests are more specifically used for the diagnosis of brucellosis, especially in Bangladesh.

### Acknowledgement

The first author is thankful to National Agricultural Technology Project-1, Department of Livestock Services, Bangladesh, for financial support to conduct this study. He is also thankful to OIE reference laboratory for brucellosis, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute (FLI), Jena, Germany.

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