

SEROPREVALENCE AND RISK FACTORS OF HUMAN BRUCELLOSIS AMONG HIGH-RISK INDIVIDUALS OF MYMENSINGH DIVISION IN BANGLADESH

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ABSTRACT

Background: Brucellosis is a common emerging and re-emerging zoonotic disease in animals and humans globally. It has drastically changed over the past decade because of various risk factors, including the drastic growth of animal husbandry, socioeconomic, political, and global trade, travel, and immigration. Domestic animals are a natural reservoir of *Brucella* spp., and animal-to-human transmission occurs through the consumption of raw milk and milk products; however, it is recognized as an occupational disease of veterinarians, animal farmers, and abattoir workers as they handle infected animals and aborted fetuses or placentae. Surveillance and epidemiology of domestic animals and humans are urgently needed to eradicate this zoonotic disease effectively nationally and globally. Although serological data on the prevalence of brucellosis in different domestic animals have been reported, studies on the seroprevalence of human brucellosis are very limited in Bangladesh.

Objective: A cross-sectional survey was conducted to estimate seroprevalence and risk factors of human brucellosis among high-risk individuals of Mymensingh.

Materials and Methods: Blood samples were collected from 182 animal handlers, and sera were separated by standard laboratory method. They were tested using the Rose Bengal plate test (RBT) and confirmed for brucellosis by enzyme-linked immunosorbent assay (i-ELISA).

Results: The overall seroprevalence of brucellosis was found to be 2.20%. Individuals over 30 years old have a higher seroprevalence of brucellosis (9.09%), while those aged 20-30 have the lowest (0.72%). Only males were found to be seropositive for brucellosis (2.5%). The study revealed that artificial inseminators had the highest prevalence of human brucellosis (10.0%), while animal owners had the lowest (5.0%). The study found that human brucellosis is most prevalent in individuals with contact durations of 10 to 20 years (6.38%), while the lowest prevalence is seen in individuals less than 10 years. The study found that the seroprevalence of human brucellosis was higher (5.80%) in individuals who consumed raw milk than those who did not.

Conclusions: This study has recorded the prevalence of brucellosis at low levels among high-risk individuals in the study area. The surveillance reports on human brucellosis are still limited in South Asia, including Bangladesh. Animals are carriers of *Brucella*, and infection in humans is often transmitted by consumption of raw milk and milk products and contact with aborted animals. Therefore, human brucellosis could be eradicated nationally and globally by eradicating animal brucellosis, which requires a 'One Health' strategy. Epidemiological surveillance and prevention of zoonotic brucellosis in South Asian countries is a great challenge due to weak interdisciplinary collaboration on the 'One Health' concept and low socio-economic status. However, avoiding risky practices like consuming raw milk and milk products and handling aborted materials without protective equipment are required, along with control of this disease in animals.

Keywords: Seroprevalence, brucellosis, animal contact people, high-risk factors, RBT, i-ELISA, Bangladesh

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INTRODUCTION

Brucellosis is a direct occupational anthroozoonosis caused by various species of *Brucella* that affects livestock, humans, and wildlife, with global significance due to its adverse impact on public health, economics, and international trade. Three *Brucella* species (*Brucella melitensis*, *B. abortus*, and *B. suis*) are highly virulent to their natural hosts and humans. They are considered endemic in most countries, predominantly endemic and neglected zoonotic diseases in developing countries of Africa and Asia.^{1,2} The annual global incidence of brucellosis in humans is approximately 2.1 million worldwide; 82.3% (144/175) of countries and 43.2% (3.2 billion/7.4 billion) of persons were considered at risk of brucellosis.² Brucellosis in animals is recognized as Bang's disease, epizootic abortion, and contagious abortion.³ Animals involved in its zoonotic transmission are goats, sheep, buffaloes, cattle, and pigs.⁴ Humans get infected through direct or indirect contact with infected animals, including handling contaminated tissues like aborted livestock placenta and ingesting contaminated animal products such as milk, meat, or carcasses.⁵ Aerosol and secretions of infected animals also act as a vehicle for human transmission.⁶ Conversely, human-to-human transmission is rare.⁷ Brucellosis is a serious occupational hazard for veterinarians, animal handlers, slaughterhouse workers, farmers, and laboratory personnel, who commonly are more exposed to animals.⁸ Human brucellosis shows various clinical manifestations, such as intermittent (undulating) fever, profuse sweating, malaise, chills, headache, weakness, arthralgia, depression, weight loss, splenomegaly, and hepatomegaly. Chronic cases may lead to arthritis, osteomyelitis, spondylitis, epididymitis, orchitis.^{2,9} In endemic areas, brucellosis is among the causes of extended-duration fever and is often categorized as a fever of unknown origin (FUO).¹⁰ *Brucella* infection occurs more predominantly in individuals having reduced levels of immunity due to stress or diseases like HIV.¹¹ The diagnostic tests mainly used for brucellosis are the Rose Bengal Test (RBT), Serum Agglutination test (SAT), Standard Tube Agglutination Test (STAT), Enzyme-linked immunosorbent assay (ELISA), and Polymerase chain reaction (PCR).^{12,13} The prevalence of brucellosis among livestock farmers, milkmen, butchers, and veterinary practitioners was reported to be 2.6% (n=386), 18.2% (n=55), 2.5% (n=40), and 5.3% (n=19), respectively.¹⁴ The prevalence of brucellosis among people with pyrexia of unknown origin was reported to be 2.0% in Bangladesh.¹⁵ During the period from 1970 to 2024, a total of 82 research articles on brucellosis were published in Bangladesh, of which 66 were in ruminants, one each in horses, pigs, and dogs, and two in milk samples, five of both animals and humans and only six in humans including one outbreak.¹⁶ Minimal reports on human brucellosis are published compared to animal brucellosis in Bangladesh.¹⁶ Brucellosis likely remains a high-risk zoonotic disease in Bangladesh, the extent to which remains poorly explored. The study highlighted the prevailing situation in the high-risk population in Mymensingh and may be utilized to develop a comprehensive strategy to prevent the spread of brucellosis in Bangladesh.

MATERIALS AND METHODS

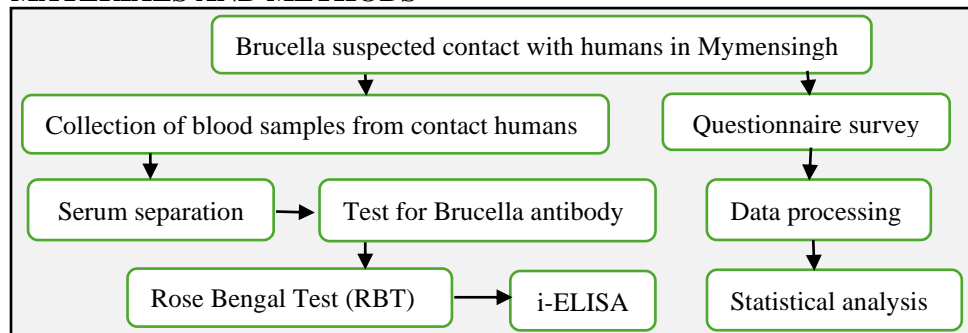


Fig. 1. Schematic representation of the experimental design

Sample collection

A total of 182 sera samples from humans were collected from different upazila of Mymensingh. Among these were 82 sera samples from animal workers, 40 sera samples from animal owners, 30 sera samples from village doctors who treat animals, 20 sera samples from inseminators, and 10 sera samples from butchers. Questionnaire data based on age, gender, disease history, duration of contact with animals, contact strategy, and raw milk eating were recorded. All the blood samples were processed for sera preparation and were tested with the Rose Bengal Test (RBT). Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA) was used for confirmatory diagnosis.

Before sampling, all contaminated glassware, including test tubes, pipettes, glass plates, vials, and agglutination plates, were disinfected in a 2% sodium hypochlorite solution. The contaminated glassware was soaked in a household dishwashing detergent solution ('Trix' Reckitt and Colman Bangladesh Ltd.) for an entire night. After brushing the glassware, it was thoroughly cleaned with running tap water, rinsed four times in distilled water, and then sterilized using either an autoclave set for 15 minutes at 121°C under 15 pounds of pressure per square inch or a dry heat method for two hours at 160°C. The autoclaved goods were dried at 50°C using a hot air oven. Autoclaving was used to sterilize micropipette tips made of disposable plastic. For later usage, all of the glassware was stored in an oven set to 50°C, and 70% alcohol spray was used as a disinfectant.

Blood and sera samples collection and preparation

At first, 5 to 7 ml blood from the radial vein of each human, with the help of a sterile disposable syringe and needle, was kept undisturbed on a tray for at least 30 min at room temperature in a slightly inclined position to facilitate clotting and separation of serum. After this period, the clotted blood samples with sera are transferred to the refrigerator at 4° C and kept overnight. Later, the sera were poured into a separate test tube from each labeled syringe, and the test tube was marked with the same number by a permanent marker. Then, the sera were centrifuged at 2500 rpm for 10 minutes. After centrifugation, a clear serum was found, and then the sera were transferred to the vial. The vial was stored at -20° C in an ice chamber until use.

Serological test

The Rose Bengal Test (RBT) was used to diagnose brucellosis. The test found a human positive and negative reactors were further confirmed by the Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA).

Rose Bengal Test (RBT)

The test was performed according to the procedure described by OIE.¹⁷ The control sera, test serum samples, and Rose Bengal antigen (INSTITUT POURQUIER-326 Rue de la Galera-34090 MONTPELLIER-FRANCE, prepared by concentrated suspension of *Brucella abortus* Weybridge stain 99) were kept for 1 hour at room temperature before beginning of the test. The test and control sera samples were homogenized using a vortex (Shaker). Thirty (30) µl of each serum to be tested was placed on a glass plate circled approximately 2 cm in diameter. Then, the antigen vial was shaken gently, and 30 µl of antigen was put beside each serum. The antigen and serum were mixed on the plate for exactly 4 minutes; the reading was taken immediately. The result was considered positive when there was any noticeable degree of agglutination.

Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA)

The assay was performed according to the protocol provided by the manufacturer (Svanova Biotech AB, Art. No. 10-2700-10, SE- 751 83 Uppsala, Sweden).

a) Preparation of PBS- Tween Buffer for I-ELISA

According to the procedure, 20x concentrate PBS-Tween solution (PBST) was diluted into 1/20 in distilled water (DW). 500 ml per plate was prepared by adding 25 ml PBST solution to 475 ml DW and mixing thoroughly.



Fig. 1. Discussion with the high-risk groups of people before collection of blood



Fig. 2. Collection of blood samples from the radial veins of humans.

b) Preparation of Anti-human IgG (H+L) AP Conjugate for I-ELISA

According to the procedure, lyophilized HLAP conjugate was reconstituted with 11.5 ml PBS-Tween buffer. Buffer was added carefully to the bottle. Then, the solution was left for one minute and mixed thoroughly. According to the recommendation, the solution was prepared immediately before use.

c) Test procedure

All reagents supplied by the manufacturer were equilibrated to room temperature 18 to 25° C (64 to 77° F) before use. 100 µl of sample dilution buffer was added to each well used for serum samples and controls. After that, 4.0µl of positive control serum (Reagent A) and 4 µl of negative control serum (Reagent B) were added to selected wells coated with *Brucella abortus* antigen. 4 µl of serum sample was added to a selected well coated with *Brucella abortus* antigen. For confirmation purposes, the samples were also run in duplicates. The plate was shaken thoroughly, and the plate/strip was sealed and incubated at 37° C (98.6° F) for 1 hour. The plates were rinsed three times with PBS-Tween buffer, and the wells were filled up at each rinse. The plate was then emptied, and the plate was tapped hard to remove all remaining fluid. Then 100 µl of HLAP conjugate was added to each well and incubated at 37 °C (98.6 °F) for 1 hour. The plate was rinsed according to the previous way. Then, 100 µl Substrate solution was added to each well and incubated for 10 minutes at room temperature 18 to 25 °C (64 to 77 °F). The beginning timing was after the first well was filled. The reaction was stopped by adding 50 µl of Stop solution to each well and mixing thoroughly. The Stop solution was added in the same order as the Substrate solution. The control and samples' optical density (OD) was measured at 450 nm in a microplate photometer. The OD was measured within 15 minutes after adding the Stop solution to prevent fluctuation in OD values.

Data processing and Statistical analysis

For interpretation, Microsoft Excel processed the questionnaire-based data statistically analyzed by IBM SPSS version 25.0.

RESULTS

Seroprevalence of human brucellosis

A total of 182 sera samples from humans were collected. Among these samples, 82 sera samples from animal workers, 40 sera samples from animal owners, 30 sera samples from village doctors, 20 sera samples from inseminators, and 10 sera samples from butchers were collected from different upazilas of Mymensingh district. The overall seroprevalence of brucellosis in contact humans with animals was 2.20%.

Risk factors associated with human brucellosis

Age

The seroprevalence of brucellosis in humans based on age has been shown in Table 1. The overall prevalence in the case of RBT and i-ELISA was 2.20% (Table 1). According to the result of the study, the highest seroprevalence of human brucellosis was found in humans above 30 years of age (9.09%). The lowest prevalence was found in humans aged between 20 to 30 years of age (0.72%) by both RBT and I-ELISA, and the prevalence was insignificantly higher in humans aged above 30 years of age than in other age groups. No positive result was found in the case of humans below 20 years of age (0%) by both RBT and I-ELISA (Table 1).

Gender

Table 1 shows the seroprevalence of brucellosis in humans based on gender. The overall prevalence in both RBT and I-ELISA was 2.20%. According to the study's results, the seroprevalence of human brucellosis was found among males (2.5%), and both RBT and I-ELISA did not find a positive result among females (0%) (Table 1).

Types of human contact

The seroprevalence of brucellosis among types of human contact has been shown in Table 4. The overall prevalence in the case of RBT and I-ELISA was 2.20%, shown in Table 4. The study found the highest seroprevalence of human brucellosis among artificial inseminators (10.0%). The lowest prevalence was found among animal owners (5.0%) by both RBT and I-ELISA, and the prevalence was insignificantly higher among artificial inseminators. RBT and I-ELISA did not find a positive result among animal workers, village doctors,

and butchers (Table 1).

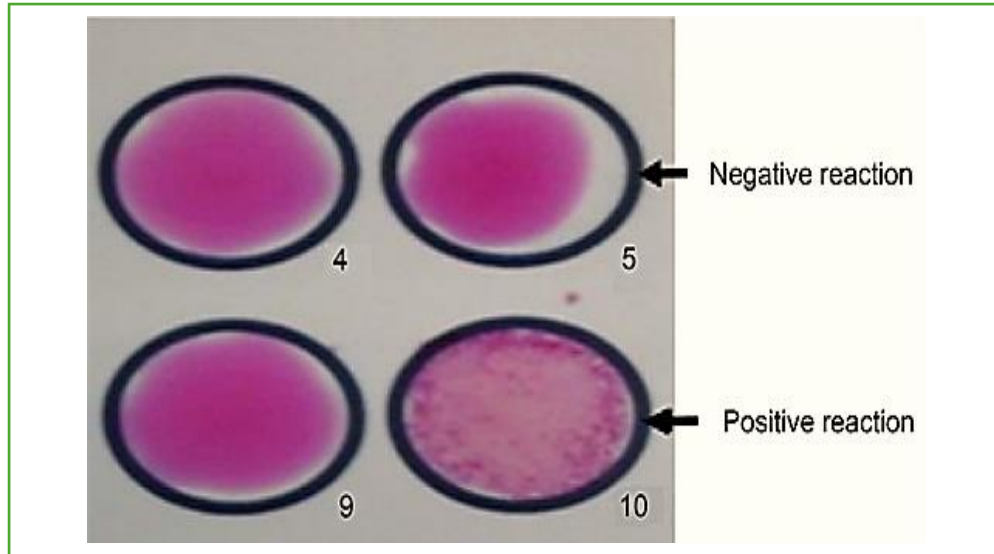


Fig. 3. Rose Bengal Test (RBT) showing Brucella positive and negative reaction in human sera

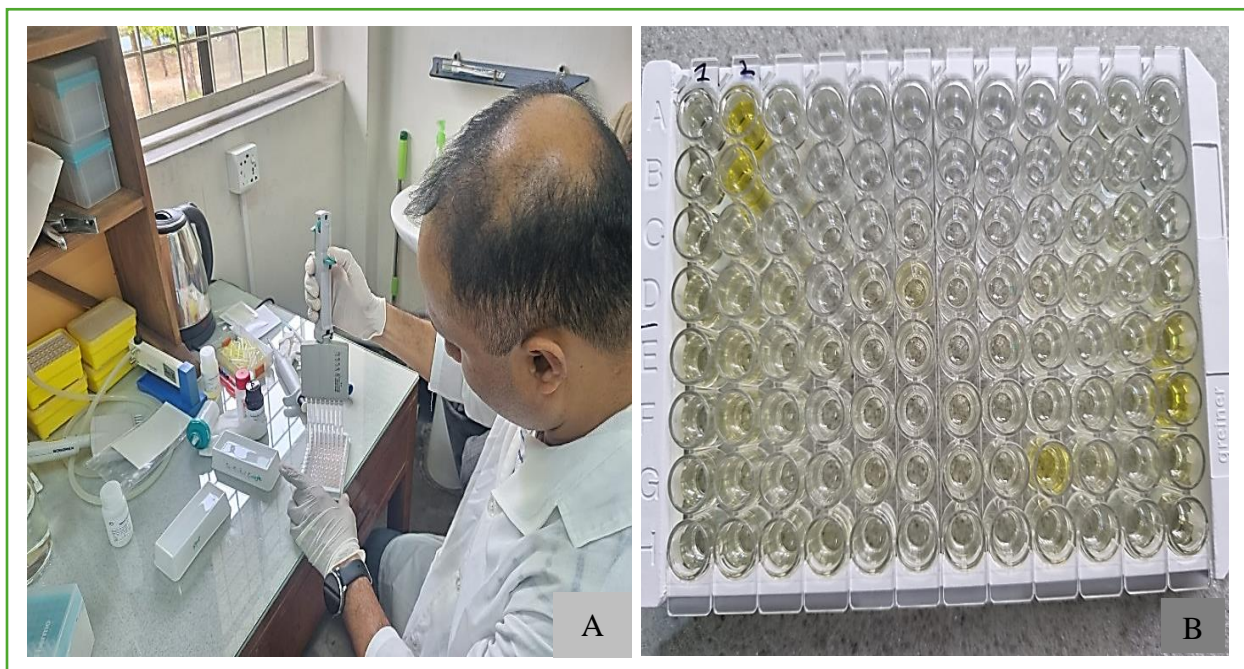


Fig. 4. Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA). A. Performing, i-ELISA and B. Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA) +ve indicates color change and -ve indicates no color change in test sera of human.

Seroprevalence and risk factors of human brucellosis

Table 1. Seroprevalence and risk factors of human brucellosis				
S/ N	Risk factors	No. of sera tested	Positive by RBT No. (%)	Positive by i-ELISA No. (%)
1.	Age (years)			
	< 20	011	0	0
	20 - 30	138	1 (0.72)	1 (0.72)
	>30	033	3 (9.09)	3 (9.09)
	Total	182	4 (2.20)	4 (2.20)
2.	Gender			
	Male	160	4 (2.50)	4 (2.50)
	Female	022	0	0
	Total	182	4.0 (2.20)	4 (2.20)
3.	Types of human contact			
	Animal workers	082	0	0
	Animal owners	040	2 (5.00)	2 (5.00)
	Village doctors	030	0	0
	Artificial inseminators	20	2 (10.0)	2 (10.0)
	Butchers	10	0	0
	Total	182	4 (2.20)	4 (2.20)
4.	Duration of human contact with animal			
	<10 years	131	1 (0.76)	1 (0.76)
	10-20 years	047	3 (6.38)	3 (6.38)
	>20 years	004	0	0
	Total	182	4 (2.20)	4 (2.20)
5.	Drinking of raw milk			
	Yes	069	4 (5.80)	4 (5.80)
	No	113	0	0
	Total	182	4 (2.20)	4 (2.20)

Duration of human contact with animal

The seroprevalence of brucellosis during human contact with animals has been shown in Table 5. The overall prevalence in the case of RBT and I-ELISA was 2.20%, shown in Table 5. According to the findings of the study, the highest seroprevalence of human brucellosis was found among humans with a duration of contact between 10-20 years (6.38%), and the lowest prevalence was found among humans with a duration of contact below 10 years (0.76%) by both RBT and I-ELISA, and the prevalence was insignificantly higher among duration between 10-20 years (Table 5). RBT and I-ELISA did not find a positive result among humans with a duration of contact above 20 years (0%) (Table 1).

Taking raw milk

Table 6 shows the seroprevalence of brucellosis in humans based on taking raw milk. The overall prevalence in both RBT and I-ELISA was 2.20%. The study's results indicate that the seroprevalence of human brucellosis was found when taking raw milk (5.80%), and there was no positive result without taking raw milk (0%) by both RBT

and I-ELISA (Table 1).

DISCUSSION

Brucellosis is a globally distributed zoonotic disease; at least 170 countries have reported human brucellosis cases. Many high-income developed countries have eradicated brucellosis in cattle but recently re-emerged. The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging.¹⁸ Re-emerging cases are frequently reported in countries where brucellosis has been controlled, like Bulgaria, Bosnia, Herzegovina, Azerbaijan, Germany, and the USA.^{19,20,21} In contrast, it remains Asia's most serious public health and socioeconomic concern, with new cases consistently reported.¹⁹ It is a high burden in several developing countries and is associated with a serious global public health and economic concern. However, it remains the most common but often neglected zoonotic disease at national and international organization levels.¹⁹ Social issues, poor husbandry practices, irregularities in the marketing and movement of domestic animals, travel, immigration, and international trade, and lack of 'One Health; activities are some of the key factors for the transmission and spread of brucellosis emerging and re-emerging and is imported from areas where it is endemic.

Brucellosis has a significant zoonotic potential, which spreads from animals to people through the ingestion of raw dairy products and by direct contact during birth and abortion. *Brucella melitensis* and *B. ovis* are primarily found in goats and sheep, while *Brucella abortus* is mainly in cattle. Both *B. abortus* and *B. melitensis* can infect people, with the latter being the most observed in human populations.²² Human brucellosis is related to the prevalence of animals and practices that expose humans to infected animals or their products. In Bangladesh, brucellosis in small ruminants and porcine caused by *B. melitensis* and *B. suis* has never been reported.^{23,24} Although many reports on brucellosis in ruminant animals have been reported, studies on human brucellosis are very limited in Bangladesh.¹⁶

The overall 2.20% seroprevalence of brucellosis in humans recorded in this study is lower than the earlier findings of review reports of 2.5 to 8.6% of humans in Bangladesh.^{25,26} Comparatively higher seroprevalence rates of 6.0%,²⁷ 9.2% IgM, and 49.2% IgG seropositivity were reported in participants of the high-risk group, and only 40.0% IgG in the animal non-risk group in Bangladesh.²⁸ However, higher seropositivity rates of 15.8% of human brucellosis in West Bengal,²⁹ 6.21% in Pakistan,³⁰ and 23.9% by RBT, 28.9% by CFT, and 31.1% by ELISA in Egypt³¹ have been reported. The lower seropositivity rate of 1.2% of human brucellosis has also been reported in Ethiopia.³² The variability in the seroprevalence of brucellosis in these reports could be attributed to differences in the management of slaughterhouses, safety standards practiced, or the investigator's technical knowledge.

Risk factors of human brucellosis

Animal caretakers, veterinarians, slaughterhouse workers, butchers, and meat and dairy product vendors are at high risk of contracting brucellosis. Acquiring infection through direct contact is a potential threat to occupational groups such as farmers, veterinarians, butchers, laboratory workers, milkers, and inseminators. *Brucella* organisms can enter the body via skin wounds, mucous membranes, or inhalation, so direct contact with infected animal tissues or fluids can be an exposure risk. Activities like carcass dressing and assisting birthing animals can increase the risk of contact with infective tissues and fluids. Higher seroprevalence rates are linked to people handling aborted fetuses and helping with abortions and deliveries of aborted fetuses without donning personal protective equipment. Handling aborted materials or attending retained placenta or dystocia without a protective globe is a common practice for most field veterinary assistants, abattoir workers, and in many rural pastoral settings in Bangladesh. Slaughterhouse workers can be infected with brucellosis by touching contaminated tissues with their bare hands, touching the conjunctiva with their contaminated fingers, and breathing in aerosol droplets. Laboratory workers are at a significantly increased risk when working with these bacterial cultures. Humans most commonly acquire *Brucella* by consuming unpasteurized dairy products, including raw milk, butter, soft cheese, or ice cream made from raw milk from infected animals. *Brucella* prefers energy from udder tissue because it is high in erythritol. Infected cows will excrete the *Brucella* germs in their milk, and ingesting unpasteurized milk is a risk

factor for human infection.³³ Significant associations of human brucellosis with human housing, contact with aborted fetuses, drinking raw milk from non-aborted and aborted, and retained placentae have been reported in Ethiopia.³²

This study has recorded 2.5% seroprevalence of brucellosis in males and 0% in females, 5.0% in animal owners and 0% in animal workers, 10.0% in artificial inseminators and 0% butchers, and 5.8% in people for drinking raw milk. These findings are not reflected with the earlier seroprevalence rates of human brucellosis reported in review articles from Bangladesh in which the seropositivity rate of 2.6% in livestock farmers, 18.6% in milkers, 2.5% in butchers and 5.3% in Veterinary practitioners, with some similarity of higher rate in males (5.6%) than females (0.8%).^{25,26} These variations could be due to the level of exposure, the brucellosis control program, differences in the study design, the study populations or groups at risk, or the participants' exposure. The lower prevalence in this study could be due to the low number of livestock, level of contact with animals, and frequency of dairy product consumption compared to the mentioned reports. The present study proves that brucellosis is a public health problem among Bangladesh's rural and urban populations.

The higher seroprevalence of brucellosis was recorded in a human above 30 years (9.09%), whereas the lowest seroprevalence rate of brucellosis in humans aged between 20 to 30 years of age (0.72%) by both RBT and i-ELISA. RBT and i-ELISA found no positive results in humans under 20 (0%). So, it may be considered that the worst affected group was young adults to adults. This finding supports the earlier inland report in which the highest seropositivity in humans was reported in 18 to 30 years of age in both the risk (60.9%) and non-risk (60.0%) groups in Bangladesh.²⁸ These findings also correlate with the highest seropositivity of 23.5% in most patients aged between 51 to 60 years old in West Bengal,²⁹ highest seroprevalence of 9.91% in human brucellosis in the 37 to 48 years age group in Pakistan,³⁰ the prevalence of this infection higher among individuals above 40 years of age in Egypt³¹ and with the highest seroprevalence of 54.47% in the 18 to 45-year-old age group in tribal and non-tribal population in an eastern state of India.³⁴ Therefore, the higher seroprevalence of brucellosis in humans above 30 years is possibly due to close association with animal husbandry activities as part of the family occupation. This may be attributed to their involvement in animal health care activities.

The seroprevalence of human brucellosis was higher in males (2.5%) than in females (0%) by RBT and I-ELISA in Bangladesh. These results support higher seropositivity of human brucellosis in males (6.94%) than in females (5.81%) in Pakistan³⁰ and males (61.44%) than females (38.56%) in India.³⁴ The higher percentage of brucellosis seropositivity in men than in women may be due to unusual men working closer to animals than females. These results suggest that male animal handlers in the study area are more at risk of brucellosis than female non-animal handlers. This may be attributed to male animal handlers being more involved with animal healthcare activities and animal management systems.

The study found the highest seroprevalence of human brucellosis among artificial inseminators (10.0%). The lowest prevalence was found among animal owners (5.0%) by both RBT and I-ELISA, and no positive result was found among animal workers, village doctors, and butchers (0%). However, those who practice artificial insemination are at high risk of getting infected by *Brucella*.³⁵ These results suggest that artificial inseminators and animal owners in the study area are more at risk of brucellosis than other groups. The prevalence of brucellosis was higher in artificial inseminators and animal owners. This may be due to unsafe handling of infected animals and materials and lack of awareness. This study also revealed no positive cases among animal workers, village doctors, and butchers, which may be due to the small number of tested samples.

The study's results indicate that the highest seroprevalence of human brucellosis was found among humans with a duration of contact between 10 and 20 years (6.38%), and the lowest prevalence was found among humans with a duration of contact below 10 years (0.76%) by both RBT and I-ELISA. These observations support the findings that most seropositive cases (6.38%) had a history of direct contact, while only 3.84% of positive humans had no contact with animals.³⁰ However, these findings contradict the higher seroprevalence of human brucellosis in humans with short duration of occupation from 0 to 5 years (63.6%) than >20 years (35.3%) duration of occupation

in Bangladesh.³⁵

The study recorded a higher seroprevalence of brucellosis (5.80%) in humans taking raw milk than in humans without (0%). These findings conform with earlier reports of higher seropositivity (55.00%) of brucellosis in humans of raw milk consumption than no raw milk consumption (46.7%) group.³⁵ The milk and milk products of various species of animals showed a high incidence of *Brucella*, especially cow milk (1.86-81.7%), buffalo milk (10.4-61.67%), camel milk (0-24%), goat milk (0-88.8%), and cheese (0-39.1%). Consuming raw milk and milk products has been identified as the leading cause of human brucellosis, with incidence rates varying from 33.9% to 100%.³⁶ The ICDDR'B scientists have identified a recent outbreak of eight confirmed cases of human brucellosis in Teknaf, Bangladesh, due to the practice of drinking raw milk by the people residing there.³⁷ This may be attributed to humans acquiring infection by consuming contaminated raw milk and milk products. This may suggest that animal health workers and rural communities are also at significant risk of contracting the disease if the disease is present in domestic animals. Further study involving a large sample size would be required for a detailed, specific analysis of the impact of this occupational disease and public health threat in Bangladesh.

Evidence showed that human exposure to *B. abortus* was widespread but unevenly distributed among the investigated occupational groups. However, there was also evidence of occupational group variation in the distribution and force of infection with *B. abortus*.

CONCLUSION

Although diagnostic and surveillance technology has advanced, brucellosis remains a serious global public health issue. The re-emerging of brucellosis is frequently reported in completely controlled countries in North America and South Europe and remains uncontrolled in low-income developing countries, including Bangladesh. The detection and surveillance of brucellosis and new technologies could help control the disease. Still, such surveillance and prevention technologies are limited to low-income countries. The high-risk group of individuals tested in this study has a low level of brucellosis. Comparatively, artificial inseminators, people consuming raw milk, and working with animals for longer tend to be more seropositive. Using personal protective equipment and avoiding drinking raw milk could decrease the risk of transmission from infected animals to humans. However, the prevention of human brucellosis requires the eradication of animal brucellosis; accordingly, eradication of animal brucellosis prevents its spread to people with a 'One Health' strategy in preventing brucellosis in humans. 'One Health' concept for surveillance investigation into the animal-human-ecosystem interface to eradicate brucellosis. Public awareness campaigns, especially animal farmers and dairy product consumers, disseminate knowledge about brucellosis and associated risk factors to prevent the disease.

Ethical Approval

All animal-related procedures and methods were carried out following the guidelines by the Animal Welfare and Experimentation Ethical Committee of the Bangladesh Agricultural University, Mymensingh (Ethical approval number - AWEEC/BAU/2023(55))

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