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Original Articles

Seroprevalence and Risk Factors for Brucellosis in a High-Risk Group of Individuals in Bangladesh

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Abstract

Brucellosis is an occupational hazard of livestock farmers, dairy workers, veterinarians, slaughterhouse workers, and laboratory personnel, all of whom are considered to belong to the high-risk occupational group (HROG). A study was undertaken to determine the seroprevalence of brucellosis, identify risk factors associated with brucellosis seropositivity, and detect *Brucella* at genus level using real-time polymerase chain reaction (PCR) among people in the HROG in the Dhaka division of Bangladesh. A sample of 500 individuals from the HROG was collected from three districts of Dhaka division of Bangladesh. A multiple random effects logistic regression model was used to identify potential risk factors. Two types of real-time PCR methods were applied to detect *Brucella* genus–specific DNA using serum from seropositive patients. The prevalence of brucellosis based on the three tests was observed to be 4.4% based on a parallel interpretation. The results of the multiple random effects logistic regression analysis with random intercept for district revealed that the odds of brucellosis seropositivity among individuals who had been in contact with livestock for more than 26 years was about 14 times higher as compared to those who had less than 5 years of contact with livestock. In addition, when the contact was with goats, the odds of brucellosis seropositivity were about 60 times higher as compared to when contact was with cattle only. Noticeable variation in brucellosis seropositivity among humans within the three districts was noted. All of the 13 individuals who tested positive for the serological tests were also positive in two types of real-time PCR using the same serum samples. Livestock farmers of brucellosis positive herds had a significantly higher probability to be seropositive for brucellosis. The study emphasized that contact with livestock, especially goats, is a significant risk factor for the transmission of brucellosis among individuals in the HROG.

Introduction

Brucellosis is an occupational hazard of livestock farmers, dairy workers, veterinarians, slaughterhouse workers, and laboratory personnel, all of whom are considered to belong to the high-risk occupational group (HROG). It is caused by bacteria of the genus *Brucella*, which manifests in different variants in different animal species. For example, *Brucella abortus* is mostly associated with cattle and *B. melitensis* with sheep, goats, and humans (Pappas *et al.*, 2005). Infection can be acquired through ingestion of unpasteurized dairy products such as soft cheeses, yogurts, and ice creams. However, direct contact with infected animals and contact with vaginal discharge, urine, feces, or blood of infected animals (especially among abattoir workers, herdsmen, veterinarians, butchers, and personnel in microbiologic laboratories) is an important transmission route. Also, *Brucella* can be transmitted through skin lesions and the mucous membrane of conjunctiva, and by inhalation of infected aerosolized particles (Wise, 1980; Young, 1983; Pappas, 2005).

Human brucellosis remains the commonest zoonotic disease worldwide, with more than 500,000 new cases reported annually (Pappas *et al.*, 2006). It is associated with a chronic debilitating infection with substantial residual disabilities. The onset of the disease may be sudden, over a period of a few days, gradual, over a period of weeks to months, or associated with non-specific symptoms that include undulating fever,

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fatigue, malaise, headache, backache, and arthralgia (Mantur *et al.*, 2007).

Human brucellosis poses major economic and public health challenges in affected countries especially in the Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico, and Central and South America (Pappas *et al.*, 2006). A limited number of studies have estimated the seroprevalence of human brucellosis in Bangladesh. These studies revealed that the prevalence of human brucellosis is 6–12.8% (Rahman *et al.*, 1983, 1988; Muhammad *et al.*, 2010). The variations in the seroprevalence reported may be due to differences in the number of samples (which ranged from 100 to 210 people in the HROG) and number of diagnostic tests used and the manner in which they were interpreted. None of these studies rigorously investigated risk factors associated with human brucellosis seropositivity despite substantial evidence that various factors—such as occupational status, consumption of unpasteurized dairy products, type of animal handled, religious background, and whether or not assisted parturition (or assisted calf birth) is practiced—influence the likelihood of brucellosis seropositivity (Abo-Shehadan *et al.*, 1996; Al-Shamahy *et al.*, 2000; Swai and Schoonman, 2009; Sofian *et al.*, 2008; John *et al.*, 2010).

The diagnosis of human brucellosis in Bangladesh has predominantly been based on serological tests namely the Rose Bengal Plate Test (RBT), Standard Tube Agglutination Test (STAT), and the Indirect Enzyme-Linked Immunosorbent Assay (iELISA), which are not gold standard tests (Rahman *et al.*, 1983, 1988; Muhammad *et al.*, 2010). These tests may not be able to differentiate between an active and a nonactive infection (Nimri, 2003). Isolation of *Brucella* spp. is the gold standard test for brucellosis. However, this is a slow process that sometimes requires Level 3 biocontainment facilities and highly skilled technical personnel, leading to high costs (Navarro *et al.*, 2004). Handling of live *Brucella* species is also associated with possible infection to laboratory personnel if biosafety rules are not strictly monitored (Yu and Nielson, 2010). Due to the speed, safety, and high sensitivity and specificity of the polymerase chain reaction (PCR), all of the positive samples based on three serological tests were subjected to PCR.

The aim of this study was to determine the seroprevalence of brucellosis, identify risk factors associated with human brucellosis seropositivity, and detect *Brucella* at the genus level using real-time PCR. The results of this study may be used to inform the development and implementation of control measures bent on sensitizing the population at risk, regulating management practices at abattoirs and farms, and abating the incidence of human brucellosis in Bangladesh.

Methods

Ethical clearance

The study protocol was peer reviewed and cleared for ethics by the Ethical Review Committee of Mymensingh Medical College. Verbal and written consents were also taken from all individuals prior to blood sample collection.

Study population and survey area

The study was carried out between September 2007 and August 2008 among livestock farmers, milkers, butchers, and veterinary practitioners in the Mymensingh, Sherpur, and Dhaka districts of Bangladesh.

In Bangladesh, about 85% of rural households own animals, and 75% of the population rely on livestock to some extent for their livelihood (www.fao.org). Livestock farmers considered for this survey were the owners or hired animal caretakers of 571 herds of Mymensingh and Sherpur districts and also workers in two government-owned farms in Dhaka District from where blood samples were taken for determining the seroprevalence of brucellosis in domestic ruminants.

Veterinary professionals at risk for brucellosis in these districts include approximately 100 individuals (approximately 25 veterinarians and animal production specialists, including their assistants) in the Department of Livestock Services (DLS) of the Bangladesh Government. Some veterinarians (actual number not known) work in Non-Governmental Organizations (NGOs) having livestock development programs. The exact number of butchers and slaughterhouse workers, as well as the actual number of milkers in these areas are not known. Estimates of butchers and milkers in these areas are 200 and 300, respectively.

Convenience samples from the population of milkers, butchers, and veterinary practitioners were obtained from Mymensingh and Dhaka districts. Milkers were selected from the Central Cattle Breeding and Dairy Farm (CCBDF) in Savar and commercial dairy farms, and those who collect and sell milk from small holder dairy farms (vendors) were also counted in this group. Butchers were selected from different locations of Mymensingh district, where a great proportion of the people are involved in this profession. Veterinary practitioners included were veterinary surgeons, veterinary field assistants, and veterinary students of Mymensingh and Dhaka districts of Bangladesh.

Questionnaire data collection

Information was collected through personal face-to-face interviews. Questionnaires recorded the following information for each subject: age, sex, address with mobile telephone number where available, level of education, occupation, type of animal handled and duration of contact in years, and previous history and presence of symptoms (pyrexia, sweating, arthralgia, backache, and headache) suggestive of brucellosis (Mantur *et al.*, 2006). The full questionnaire is available upon request from the corresponding author.

Collection and handling of blood samples

About 4 mL of blood was collected with disposable needles and Venoject tubes, labeled, and transported to the laboratory on ice (after clotting) within 12 h of collection. Blood samples were kept in the refrigerator (2–8°C) in the laboratory, and 1 day later sera were separated by centrifuging at $6000 \times g$ for 10 min. Each serum was labeled to identify the individual and stored at −20°C. Each serum was divided into two tubes, each containing about 1 mL of serum. One aliquot was used for testing, and the other was preserved in a serum bank. Among the total of 500 individuals considered to be in the HROG, 386 were livestock farmers. The serological status of these farmers was compared with that of the herd they managed.

Serological tests

All blood samples were tested in parallel using the RBT, STAT, and iELISA in the Medicine Department laboratory of Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. The tests are briefly described next.

Rose Bengal Test (RBT). RBT was performed according to standard procedure (Alton *et al.,* 1988). Briefly, sufficient antigen, test sera, and positive and negative control sera for a day's testing were removed from refrigeration and brought to room temperature (22 ± 4°C). Equal volumes (30 μL) of serum and antigen (concentrated suspension of *B. abortus* biotype 1 [Weybridge 99]; Institut Pourquier, Montpellier, France) were mixed and rotated on a glass plate for 4 min. The result was considered positive when agglutination was noticeable after this delay.

Standard Tube Agglutination Test (STAT). STAT was carried out on doubling dilution of serum from 1:20 to 320 according to standard procedure (Alton *et al.,* 1988). *Brucella abortus* antigen (Cypress Diagnostics, Langdorp, Belgium) was used according to the instruction of the manufacturer. The test tubes were incubated at 37°C for 24 h. Positive reactions were determined by observing agglutination in 1:160 or more dilution of test serum.

Indirect Enzyme-Linked Immunosorbent Assay (iELISA). iELISA was performed according to Limet et al. (1988) using *B. abortus* biotype 1 (Weybridge 99) as antigen. For the standard curve, six dilutions (1/270 to 1/8640) of the positive reference serum (no. 1121) were prepared. Fifty microliter of serum dilutions (1:50 in buffer consisting of 0.1M glycine, 0.17M sodium chloride, 50mM EDTA, 0.1% (volume) Tween 80, and distilled water, pH 9.2) were added to the wells in duplicate. The plates were incubated for 1 h at room temperature. Binding antibodies were detected using a Protein G-horseradish peroxidise (G-HRP) conjugate as described by Saegerman *et al.* (2004). Citrate-phosphate buffer containing 0.4% *O*-phenylenediamene and 2 mM H_2O_2 was used to visualize the peroxidase activity. Reading of optical densities (OD) was done at 492 nm and 620 nm using VMax® Microplate Reader. The results (OD_{492} –OD_{620}) were expressed as antibody units in comparison with a reference serum. The conversion of ODs into units (U/mL) was done using six dilutions of the reference serum to establish a standard curve. The cut-off value for a positive result was defined at 20 U/mL of test serum.

Real-time PCR

Real-time PCR was used to detect *Brucella* spp., mainly *B. abortus* and *B. melitensis.* DNA was isolated from 13 sera that tested positive on all three serological tests. About 200 μl of serum was used for extraction of DNA from sera using DNeasy spin columns (Qiagen Inc., Valencia, CA) according to the manufacturer's recommendations. The most frequently described PCR target for the diagnosis of human brucellosis is the *bcsp31* gene encoding a 31-kDa antigen conserved among *Brucella* spp. (Navarro *et al.,* 2004). The BCSP31-PCR assay was carried out using standard procedure (Baily *et al.,* 1992; Bounaadja *et al.,* 2009), and IS711-PCR was done using the procedure described by Halling *et al.* (1993). The cut off for the cycle threshold (Ct) values of real-time PCR positivity is 40. Above this threshold, the sample is considered negative and below the threshold it is considered positive. The real-time PCR assay was performed at the National Reference Centre for Brucellosis, Veterinary and Agrochemical Research Centre (CODA-CERVA) in Belgium.

Statistical analysis

To determine the potential risk factors associated with human brucellosis sero-positivity, individuals were considered positive if they tested positive in at least one serological test along with the presence of any of the clinical symptoms suggestive of brucellosis as mentioned in questionnaire's data collection section.

Firstly, a univariate analysis was performed using a random effects logistic regression model. The model uses, as response, the brucellosis status of the individuals and each risk factor or indicator variable in turn as the independent variable. Occupational status was forced into the model as it is of primary interest. The possible effects of variations in brucellosis seropositivity among districts were accounted for by incorporating district as a random effect in the model (Van-Leeuwen *et al.,* 2010).

Variables with a p-value ≤ of 0.10 in the univariate analysis were further analyzed in a multivariable random effects logistic regression model. A manual forward stepwise model building approach was employed with the Akaike's Information Criterion (AIC) as the calibrating parameter to select the final model. In this approach, the best univariate model is selected as the model with the lowest AIC value. The remaining variables are then added each in turn to form three variable models. The best three-variable model is selected based on the AIC. This is repeated until the addition of one more variable fails to improve the model fit; in other words if the AIC does not change or starts to increase. The model with the smallest AIC is considered to be the most appropriate model. The effects of confounding were investigated by observing the change in the estimated coefficients of the variables that remain in the final model once a non-selected variable is included. When the inclusion of a non-significant variable led to a change of more than 25% of any parameter estimate, that variable was considered to be a confounder and was included in the model. All two-way interaction terms of the variables remaining in the final model were assessed for significance based on the AIC values, i.e., comparing the AIC values of the model with the desired interaction term and the corresponding model with no interaction terms (Dohoo *et al.,* 2003).

The intra-class correlation coefficient (ICC), which is a measure of the degree of clustering of individuals belonging to the same district, was computed. In random effects logistic regression models, the individual level variance δ^2 on the logit scale is usually assumed to be fixed to $\pi^2/3$ (Snijders and Bosker, 1999). The variability attributed to differences among districts is given by:

$$ICC_{DISTRICT} = \delta^2_{INT:DISTRICT}/(\delta^2_{INT:DISTRICT} + \pi^2/3)$$

If the ICC is zero, it implies that there is no variability in brucellosis seropositivity among districts but rather a higher variability among humans within districts.

The models were built using the xtmelogit () function in STATA, version 11, software (StataCorp LP, College Station, TX). Model selection was done using Laplacian approximation, whereas parameter estimates from the final model were obtained using Adaptive Gaussian Quadrature (Twisck, 2003). The robustness of the final model was assessed by increasing the number of quadrature (integration) points and monitoring changes in parameter estimates (Franken *et al.*, 2009).

Results

Descriptive statistics

There were a total of 500 individuals from the the Mymensingh, Sherpur, and Dhaka districts of Bangladesh. The prevalence of brucellosis based on the three tests was observed to be 4.4% following a parallel interpretation of the three tests. The prevalence of brucellosis for each category of each of the factors considered is presented in Table 1. The prevalence was found to be highest (28.3%) among individuals who indicated symptoms linked to brucellosis. The prevalence of brucellosis was also found to be higher among milkers as compared to livestock farmers, butchers and veterinary practitioners. The prevalence of brucellosis appeared to be higher among individuals who handled only goats; the prevalence was found to be higher with increased duration of contact with animals. The prevalence of brucellosis among males was higher (5.6%) compared to that of females (0.8%). Finally, among those who consumed raw milk, the prevalence was higher (11.4%) as compared to those who did not consume raw milk (3.9%). Out of 571 herds, 386 people of 337 (59.0%) herds agreed to provide blood samples.

Table 1. Potential Risk Factors Associated with Household Level Seroprevalence of Brucellosis Based on a Univariate Random Effects Model

Factor	*Tested*	*Positive*	*Prevalence*	*95% CI*
Age group (years)				
14–20	44	1	2.3	(0.06, 12.0)
21–40	231	7	3.0	(1.2, 6.1)
41–80	225	14	6.2	(3.4, 10.2)
District				
Dhaka	63	12	19.0	(10.2, 30.9)
Mymensingh	410	10	2.4	(1.2, 4.4)
Sherpur	27	0	0	(0, 12.8)[a]
Education				
None to secondary	468	22	4.7	(3.0, 7.0)
College to university	32	0	0.0	(0, 10.9)[a]
Sex[b]				
Female	125	1	0.8	(0.02, 4.4)
Male	375	21	5.6	(3.5, 8.4)
Occupation				
Livestock farmer	386	10	2.6	(1.2, 4.7)
Milker	55	10	18.2	(9.1, 30.1)
Butcher	40	1	2.5	(0.06, 13.2)
Veterinary practitioner	19	1	5.3	(0.1, 26.0)
Duration of contact with animals (years)[c]				
0.08–5	169	1	0.59	(0.01, 3.3)
6–15	166	3	1.8	(0.4, 5.2)
16–25	91	6	6.6	(2.5, 13.8)
≥26	76	14	16.2	(8.7, 26.6)
Type of animal handled[c]				
Cattle only	343	12	3.5	(1.8, 6.0)
Cattle and goat	86	4	4.7	(1.3, 11.5)
Goat	71	6	8.5	(3.2, 17.5)
Drinking raw milk				
No	465	18	3.9	(2.3, 6.0)
Yes	35	4	11.4	(3.2, 26.7)
Symptoms[c]				
No	440	5	1.1	(0.4, 2.7)
Yes	60	17	28.3	(17.5, 41.4)

[a]Exact binomial confidence interval.

[b]Significant at 10% but not at 5% so was considered as a potential risk factor or indicator variable and therefore included in the multivariable random effects logistic regression model.

[c]Highly significant ($p < 0.001$).

CI, confidence interval.

Factors associated with brucellosis seropositivity in humans based on a univariate analysis

The results of the univariate random effects logistic regression analysis with occupation forced into the model and a random intercept for district revealed that, type of animal handled, and duration of contact with animals were highly significantly associated with human brucellosis seropositivity ($p < 0.05$; Table 1). On the other hand, gender was not significant at the 5% level, but since its p-value was ≤ 0.10, it was considered as a potential risk factor and was thus included in the multivariable random effects model.

Multiple random effect logistic regression model

Out of the potential risk factors initially considered in the multiple random effects logistic regression model, four were included in the final model. None of the two-way interaction terms were statistically significant ($p > 0.05$). Gender appeared to be a confounding variable and was therefore included in the model. Increasing the number of quadrature points had no influence on the estimated fixed effects and the variance component parameters indicating that the model is robust. The estimated odds ratios (ORs) and their 95% confidence intervals (CIs) are presented in Table 2.

The variance component of the model with no covariates yielded an ICC of 0.28. This implies that 28% of the variance in the log odds of brucellosis seropositivity is attributed to differences among districts. After incorporating the significant risk factors, the ICC for districts remained almost the same at 0.27. The between-district variability of 27% suggests that there is a weak variability in human brucellosis cases among districts in Bangladesh but a high between-human variability within districts.

From the final model (Table 2), it can be seen that for those people who owned or handled mainly goats, the odds of brucellosis seropositivity were significantly higher than those of people who handled only cattle (OR = 59.8, $p < 0.001$). Also though, not statistically significant, relative to those who owned or handled only cattle, those who handled cattle and goats were 9.5 times more likely to be brucellosis seropositive. The odds of human brucellosis seropositivity increased significantly with an increase in the duration of contact with animals. In fact, for individuals who had been working with livestock for more than 26 years, the odds of brucellosis seropositivity were significantly higher compared to those who had been working for less than 26 years (OR = 14.2, $p = 0.02$).

Results of the real-time PCR

The findings from the real-time PCR for the seropositive cases are shown in Table 3. All of the 13 positive human cases based on the three tests were positive in both PCR. The mean Ct values of BCSP31 and IS711 real-time PCR test were 37.03 and 34.40, respectively, indicating a positive reaction in both situations.

The relationship between brucellosis-positive animal herds and occurrence of human infection is shown in Table 4. Livestock farmers of brucellosis-positive herds had significantly higher odds to be infected (OR = 10.2; 95% CI: 2.8–37.1).

Discussion

The present study represents the first report on the risk factors for brucellosis among individuals in high-risk occupations in Bangladesh. The results of this study suggest that the presence of brucellosis-related symptoms, type of animals owned or handled, and duration of contact with animals are highly significantly associated with brucellosis seropositivity in humans in the HORG in the Mymensingh, Sherpur, and Dhaka districts of Bangladesh. In addition, there is considerable variability in brucellosis seropositivity among humans

Table 2. Final Model of Risk Factors Associated with Human Brucellosis Seropositivity Among 500 People at High Risk for Brucellosis Within the Mymensingh, Sherpur, and Dhaka Districts of Bangladesh

Risk factors	*OR*	P-*value*	*95% CI*
Occupational status			
Butcher	1	—	—
Livestock farmer	2.8	0.384	(0.28, 26.94)
Milker	16.9	0.053	(0.99, 293.85)
Veterinary practitioner	3.7	0.468	(0.11, 122.59)
Animal handled			
Cattle only	1	—	—
Cattle and goat	9.5	0.053	(0.97, 98.83)
Goat	59.8	<0.001	(6.40, 559.93)
Duration of contact with animals (years)			
0.08–5	1	—	—
6–15	2.6	0.427	(0.24, 28.43)
16–25	9.9	0.047	(1.03, 95.30)
≥26	14.2	0.019	(1.56, 129.6)
Sex			
Women	1	—	—
Men	6.2	0.120	(0.62, 60.98)
Variance components	Estimate	SE	
District	1.22	0.81	(0.34, 4.46)

OR, odds ratio; SE, standard error; CI, confidence interval.

Table 3. Real Time Polymerase Chain Reaction (PCR) Confirmation of Seropositive Patients

			Ct values		
PCR type	*Tested*	*Positive*	*Mean ± SE*	*Minimum*	*Maximum*
BCSP31	13	13	37.03 ± 0.46	33.2	39.4
IS711	13	13	34.40 ± 0.44	31.0	36.0

Ct, cycle threshold; SE, standard error.

within the three districts. These variations may be due to heterogenous distribution of different HROG people in different districts. In Mymensingh District, all type of HROG people were sampled; in Dhaka the sample was predominantly composed of milkers; and in Sherpur, all people sampled were livestock farmers. It was observed from this study that milkers have relatively higher brucellosis seroprevalence than livestock farmers, butchers, and veterinary practitioners. These factors may explain the variability of brucellosis seropositivity among individuals within district.

The prevalence of brucellosis in the HROG based on parallel interpretation of the three tests was observed to be 4.4%. This seroprevalence is comparable to those of other reports from this area (Muhammad *et al.*, 2010; Thakur and Thapliyal, 2002). Brucellosis in humans in Bangladesh is ignored, misdiagnosed, and thought to have very low sporadic incidence. The findings of this study reveal that brucellosis among people in the HROG is not uncommon. In this study, about 28.5-fold increased odds of infection was found in HROG individuals having clinical symptoms suggestive of brucellosis. So, medical doctors should use these findings as a diagnostic clue in HROG individuals for brucellosis (Araj and Azzam, 1996).

The duration of contact with animals was found to be strongly associated with human brucellosis seropositivity. This finding is consistent with results from other studies (Rahman *et al.*, 1983; Abo-Shehada *et al.*, 1996). This could be due to long-term cumulative exposure by individuals to brucellosis-infected livestock or to a contaminated environment (which increases the chance of getting infected).

It was observed in this study that about 14.2% (55/386) livestock farmers shared same premises with animals, and the majority (29/55) of livestock species kept are goats. The relatively low socioeconomic status of the farmers makes it impossible to build separate animal houses for protection from predators, especially in the case of small ruminants. Among those who keep goats inside their houses, the seroprevalence of brucellosis was 6.9%. This finding of very intimate contact with goats may explain the relatively higher seroprevalence of brucellosis in HROG individuals having contact with goats. Similar observations were also made by other authors (Rahman *et al.*, 1988; Omer *et al.*, 2002).

Table 4. Relationship Between Brucellosis Seropositivity Status of Livestock Herds (Involving Cattle, Sheep, and Goats) and Human Brucellosis Seropositivity

	Livestock farmers			
Herd status	*Tested*	*Positive*	*Prevalence*	*95% CI*
Negative	309	3	0.1	(0.2, 2.8)
Positive	77	7	9.1	(3.7, 17.8)

CI, confidence interval.

Brucellosis is an occupational disease in livestock farmers, dairy workers, butchers, veterinarians, and laboratory personnel. For this reason, occupational status was forced into the final model. The odds of brucellosis seropositivity appeared to be high for milkers (OR = 16.9), which was consistent with findings from other studies (Rahman *et al.*, 1983; Omer *et al.*, 2002). Among dairy farm workers, undulant fever seems to be almost (but not entirely) limited to those who handle and milk the cows. The higher seroprevalence in milkers confirms the impression that intimate contact with animals is more important than consumption of infected milk (McDevitt, 1971).

Even though gender was an important confounding variable in this study, its non-significance as a risk factor for brucellosis seropositivity in this study may be explained by the very low proportion of brucellosis seropositive cases among females (one out of 25). Males were apparently about six times more likely to be brucellosis seropositive as compared to females. This is because the occupations described in this study are male dominated in Bangladesh. Several other studies have indicated gender as significant risk factors for brucellosis (Wassif *et al.*, 1992; Shehata *et al.*, 2001; Mantur *et al.*, 2004; Meky *et al.*, 2007).

In other studies, the consumption of raw milk has been shown to be the most significant risk factor for the transmission of brucellosis among humans (Godfroid *et al.*, 2011). However, in this study, consumption of unpasteurized dairy products was not a significant risk factor. This is probably due to the fact that our study subjects are limited to those in the HROG, most of whom are not the main consumers of the finished dairy products. To investigate the role of consumption of unpasteurized dairy products, a study should be performed that covers the entire population and not only those people in the HROG.

A total of 13 individuals from the HROG were positive in all three serological tests. From the results of both real-time PCR methods, *Brucella* genus–specific DNAs were detected in all of those 13 seropositive cases. This indicates that, among the test positive cases, there were no false positives. The detection of *Brucella* genus specific–DNA using real-time PCR from human sera is in agreement with findings from other studies (Zerva *et al.*, 2001; Debeaumont *et al.*, 2005; Queipo-Ortuno *et al.*, 2005). Detection of *Brucella* genus–specific DNA using real-time PCR is a rapid, highly sensitive, specific, and not hazardous test for laboratory personnel, which can be used as a better alternative to culture. At least at the regional level, a laboratory can be established with the facilities for performing serum-based real-time PCR. This will assist in the confirmation of the disease in the HROGs having signs of brucellosis. It can be added here that all of the 13 seropositive patients were treated with a combination of doxycycline and rifampicin, which successfully cured them, except for one relapse case (data not shown).

Working in a brucellosis-positive herd would normally increase the probability of getting infected with brucellosis. In this study, this risk was quantified as 10 times more likely for the livestock farmers having at least one seropositive animal in their herds.

This is one of the first studies that rigorously investigated and quantified risk factors for brucellosis seropositivity in Bangladesh using a random effects logistic regression model. The advantage of such a modeling approach is that it accounted for clustering of individuals within districts. However, the limitation is that samples of milkers, butchers, and veterinary practitioners are convenience samples generated by the use of nonprobabilistic sampling methods, which has the effect of limiting the generalization of the results to the entire, at-risk Bangladesh population. Given the unavailability of a sampling frame, randomness of the sample from these groups of individuals is almost impossible. Moreover, such a study is based on the contentment of patients, and it is difficult to evince this constraint.

Evidence from this study on risk factors for brucellosis seropositivity in humans can be strengthened by increasing the number of samples and ensuring a more representative sample including milkers, butchers, and veterinary practitioners. The large odds ratios with wide CIs obtained in our study should be cautiously interpreted, given that the distribution of the individuals within the different categories of the risk factors was not even and the frequencies were sometimes very low.

In conclusion, our study revealed that the duration of contact with animals and the type of animal handled appeared to be the most significant risk factors for human brucellosis seropositivity in the Mymensingh, Sherpur, and Dhaka districts of Bangladesh. These two factors can be easily altered by educating individuals at HROG on the potential risks of extensive contact with livestock. The non-existence of a vaccine against brucellosis in humans or the difficulty of accessing a safe and efficacious vaccine implies that controlling this zoonotic disease in animals will directly lead to prevention in humans (especially with respect to biosecurity). The significant risk factors identified in this study can be regarded as proxies for many other management factors that were not included in the questionnaire. Intervention studies will therefore be needed to confirm the role of these factors on human brucellosis seropositivity.

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Disclosure Statement

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