Brucellosis among High-Risk Occupational Population at Sylhet City of Bangladesh

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Abstract

Background: Brucellosis is a worldwide zoonotic disease. The occurrence in humans is usually associated with occupational or domestic exposure to infect animals or their product. It is a major public health concern worldwide. **Objective:** This study was conducted to estimate the seroprevalence and factors associated with human brucellosis. **Methodology:** This was a cross-sectional study. Data was collected during the period of July 2016 to June 2017. Participants who were in high-risk professionals were selected using simple random sampling technique. Data were collected using a structured questionnaire and analyzed for anti-Brucella IgG and IgM in the serum were determine by Enzyme Linked Immunosorbent Assay (ELISA) technique. **Results:** A total of 90 participants among which 65 participants were high risk professionals were recruited for this study. The seroprevalence of anti-Brucella IgG was found to be 32 (49.2%). Concerning occupation, the highest seropositivity was seen in dairy farm workers 3(16.7%). According to type of animal handled highest seropositivity was found 9 (56.3%) in 16 participants of both cow and goat handlers. There was no statistically significant association between seropositivity and type of animal handled, duration of occupation (p>0.05). The participants with history of raw milk consumption seropositivity were seen in 11(55.0%) cases. Participants having symptoms suggestive of brucellosis showed seropositivity in 13(59.1%) cases. **Conclusion:** In conclusion our study revealed that the brucellosis is still an occupational health hazard in Bangladesh. Consumption of raw dairy milk appeared to be one of the most important risk factors for human brucellosis.

Keywords: Brucellosis; occupational hazard; risk factor; seroprevalence; zoonosis; Bangladesh

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Introduction

Human brucellosis is a true zoonosis caused by organism of the genus Brucella. The disease mainly occurs in various domestic animals like cattle, sheep, goat or pig and wild animals as well as humans[1]. Humans are infected by direct contact with infected animals or animals product[2]. Brucellosis is mainly an occupational disease affecting farmers, shepherds, abattoir workers, laboratory workers, veterinarians and slaughterhouse workers due to frequent exposure with animals are a greater risk of acquiring Brucellosis[3]. Splashing of infected fluid in the conjunctiva and inhalation of Brucella has been reported in slaughterhouse workers where the concentration of Brucella can be high due to aerosol generation[4]. Consumption of undercooked meat, unpasteurized dairy products especially raw milk is the most common means of transmission[5].

Brucellosis is an ancient and one of the widespread

zoonotic diseases. Since 1920, in addition to Brucella melitensis, Brucella abortus and Brucella suis at least eleven new species have been identified as belonging to the Brucella genus1. Each one with individual host preferences, epidemiology, and pathogenicity. B. abortus infect cattle, Brucella melitensis infect sheep and goats, B. suis infect pigs[6]. Animal brucellosis is characterized by reproductive failure resulting in abortion, still birth and reduce milk yield[7]. Human brucellosis usually present acute or subacute febrile illness, which is undulant pattern. Major clinical feature fever (78.7%), myalgia (66.0%), arthralgia (34.0%), and low back pain (45.0%)[8]. Acute febrile condition resembles with malaria, typhoid fever, so it is a diagnostic challenge in malaria-endemic areas[9]. Osteoarticular involvement is the most frequent complication of brucellosis[10]. Severe complications of brucellosis infection are not rare, hepatomegaly reported in 15.0% to 20.0%, the central nervous system is involved in 5.0% to 7.0% and 0.0% to 2.0% of patient manifest as endocarditis[11]. One in 10 men suffers from epididymo-orchitis[12].

Brucellosis is a major public health significance with more than five hundred thousand new human cases are reported annually10. Worldwide, reported incidence of human brucellosis in endemic disease areas varies widely, from less than 0.01 to more than 200 per 100,000 population[13]. The disease is endemic in the Mediterranean region, the Middle east, Latin American, Africa and part of Asia and yet it is often unrecognized and frequency goes unreported[14]. There are a limited number of studies have estimated of human brucellosis in Bangladesh. These studies revealed that the prevalence of human brucellosis is in 6.0% to 12.8% cases[15]. The occurrence of brucellosis in India was first established early in the previous century and since then has been reported from almost all states[16]. Several publications from India revealed that the prevalence was 41.23% in veterinary inspectors, 2.06% in shepherds and 1.03% in butchers[17]. These publications indicate that human brucellosis can be a fairly common disease in India. There are few studies of brucellosis in animal and human in Bangladesh. Prevalence of brucellosis varied based on occupational people (2.5% to 18.6%) and species of domestic animals (3.7% in cattle, 4.0% in buffalo, 3.6% in goat, and 7.3% in sheep). These observation supports that brucellosis is an occupational health hazard in Bangladesh among milkers, farmers, and veterinarians. The type of animals handled, duration of contact with domestic animals and consumption of raw milk are the risk factors associated with human brucellosis in Bangladesh[18]. Thus, this study was conducted to estimate the seroprevalence and factors associated with human brucellosis.

Methodology

Study Settings and Population: This cross-sectional study was investigated in the Department of Microbiology at Sylhet MAG Osmani Medical College, Sylhet, Bangladesh during July 2016 to June 2017 for a period of one year. This study targeted the high-risk occupational population of Brucellosis such as slaughterhouse, meat-shop, dairy farm workers and veterinary practitioners in the Sylhet district of Bangladesh. There is an animal slaughterhouse under Sylhet city corporation. Approximately 15 individuals are working there. Several slaughterhouse workers work in various butcher's shop. According to the chart of city corporation there are a total of 54 meat shop in Sylhet city. The estimated number of employees in the sale center is 80. Thus, a great proportion of the people are involved in this profession. Dairy farm facility is located in a Sylhet city. The actual number of dairy farm workers are not known. Veterinary professionals are very high risk for brucellosis. In Sylhet city there are District veterinary hospital and District artificial insemination center in the department of livestock services (DLS) of the Bangladesh Government. These occupational high-risk persons were the study population of this research work. People who are not willing to participate were excluded from this study.

Study Procedure: Among them 65 sample population were High risk occupational population. Sampling technique was simple random sampling. After selection of study population all members were given individual identification numbers. Participants of sample population were selected through lottery by hand. Data were collected in a preformed data collection sheet. All the participants gave informed written consent to participate in this study. The participants were asked to complete a questionnaire consisted of question about their demographic data (Age, Socioeconomic condition, Occupation).

Laboratory Procedure: With all aseptic precaution 3 ml of venous blood were collected from each participant by venipuncture. Samples were taken into sterile test tubes and allowed to clot at room temperature for about 30 minutes. Serum was separated by centrifuging at 2000 rpm for 10 minutes and transferred to a Eppendorf tube with proper

labelling. Serum was stored at -20ºC until further analysis. The sera were analyzed using Enzyme Linked Immunosorbent Assay technique for Anti-Brucella IgM and IgG. Manufacturer of the reagent: CALBIOTECH A life science company, CA 92020 U.S.A. Catalog # BA053M, Lot no: BAM5028, Kit name: BrucellaIgM. Catalog # BA052G, Lot no: BAG5124, Kit name: BrucellaIgG. These kits were used for the qualitative determination of Anti-Brucella IgM and IgG.

Statistical Analysis: Sample size was calculated considering the prevalence of human brucellosis in Dhaka district 24% with 5% significance level and 10% marginal error. Sample size was calculated by using the Guilford and Frucher's formula. The calculated sample size was 70. For better evaluation of seroprevalence sample size taken was 90. Data were processed and analyzed with the help of SPSS (Statistical Package for Social Sciences) Version 21.0. Association between variables has been analyzed using chi-square analysis and presented along with frequency and percentages. The logistic regression model was used to study the association between exposure variables and seroprevalence of brucellosis. The association of a particular variable was expressed as odd ratio (OR) with a 95% confidence interval (Cl). A probability value (p) $\leq$ 0.05 was considered statistically significant.

Ethical Clearance: Ethical clearance was obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College beforehand. All the ethical committee guidelines were followed during the conduction of the study.

Results
There were a total of 65 participants of high risk professionals were involved in this study. All the participants were male. The maximum (35.4%) of the participants were in the age group of up to 30 years. With regard, to socioeconomic status majority 31
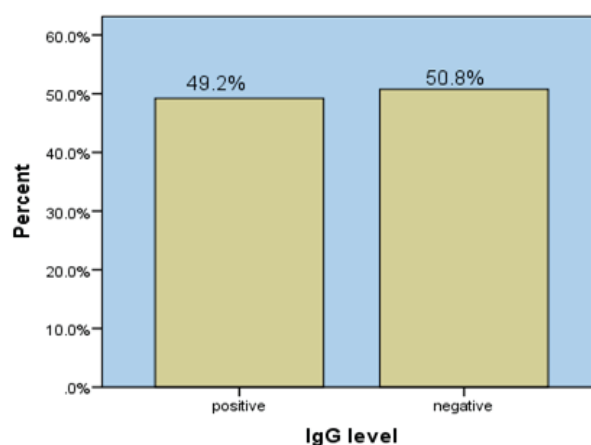

Figure I: Immunological status of the participants

Table 1: Demographic characteristics associated with Brucella seroreactivity among high risk population in Sylhet city

Variables	IgG Level		Total	P value
	Positive	**Negative**		
Socioeconomic Condition				
• Poor	14(45.2%)	17(54.8%)	31(100.0%	>0.05
• Lower Middle Class	13(54.2%)	11(45.8%)	24(100.0%)	
• Middle Class	5(62.5%)	3 (37.5%)	8(100.0%)	
• Upper Middle Class	0(0.0%)	2(100%)	2(100.0%)	
Duration of Occupation				
• 0 to 5 Years	7(63.6%)	4(36.4%)	11(100%)	>0.05
• 6 to 10 Years	8(53.3%)	7(46.7%)	15(100%)	
• 11 to 20 Years	11(50.0%)	11(50.0%)	22(100%)	
• More than 21 Years	6(35.3%)	11(64.7%)	17(100%)	
Animal Handled				
• Cow	20(54.1%)	17(45.9%)	37(100%)	>0.05
• Goat	3(25.0%)	9(75.0%)	12(100%)	
• Both cow and goat	9(56.3%)	7(43.8%)	16(100%)	
Raw Milk Consumption				
• Yes	11(55.0%)	9(45.0%)	20(100%)	>0.05
• No	21(46.7%)	24(53.3%)	45(100%)	
Symptom				
• Yes	13(59.1%)	9(40.9%)	22(100%)	>0.05
• No	19(44.2%)	24(55.8%)	43(100%)	

(47.7%) of the participants were poor out of 65 participants. According to occupational status (n=65), slaughterhouse workers 16(25.0%), meat-shop workers 19 (29.0%), dairy farm workers 18 (28.0%) and veterinarians 12 (18.0%). Majority of the participants 39 (60%) gave history being in the same occupation for more than 11 years. The analysis of their blood sample using ELISA technique showed that Brucella IgG seropositivity was found 32 (49.2%) of the participants.

Socioeconomic condition of the participants had divided into four groups. Participants with middle class showed highest seropositivity 5 (62.5%). Participants with 0-to-5-year duration of job had a highest seropositivity 7(63.6%). Regarding animal handlers the highest 9(56.3%) IgG seropositivity was found in 16 participants of both cow and goat handlers. The seropositivity was found to be higher (59.1%) among individuals who indicated symptoms linked to brucellosis. Finally, among those who consumed raw milk, the seropositivity was higher (55.0%) as compared to those who did not consume raw milk (46.7%). There was no significances association between Brucella IgG seropositivity and type of animal handled, symptom and raw milk consumption (p>0.05) (Table 1).

According to the occupational status the highest seropositivity was found in dairy farm workers 3 (16.7%) followed by meat-shop workers 2 (10.5%) and veterinarians 1(8.3%). No seropositivity was seen in slaughterhouse workers 0 (0.0%). There was no significant association between Brucella IgM seropositivity and occupation of the participants (p>0.05) (Table 2).

Table 2: Seropositivity by Occupation of the Participant

Occupation	IgM level		Total	P value*
	Positive	Negative		
Meat Shop Worker	2(10.5%)	17(89.5%)	19(100%)	
Slaughterhouse Worker	0(0.0%)	16(100%)	16(100%)	
Dairy Farm Worker	3(16.7%)	15(83.3%)	18(100%)	0.413
Veterinarian	1(8.3%)	11(91.7%)	12(100%)	
Total	**6(9.2%)**	**59(90.8%)**		

Chi-square test was performed to see the level of significance

Discussion

This study was designed to see the risk factors related with brucellosis for high-risk professionals in Sylhet city. Here slaughterhouse workers, meat-shop workers, dairy farm workers and veterinarians were selected as high risk professionals[17].

In this study regarding occupational group the maximum Brucella seropositivity was found in dairy farm workers (16.7%) (n=18). Study conducted by Mahmud et al[19] from Bangladesh revealed that the 6.45% seropositivity was found among dairy farm worker (n=31) that was lower than our study. Study from Mexico conducted by Hernandez et al[20] reported higher seropositivity among dairy farm workers (29.3%, n=58). The dairy farm workers are professionally in very close contact with animals. Diseased animals excreted huge amount of brucella through amniotic fluid during parturition and also large amount of brucella are excrete through milking. Consequently, these are the risk factors for developing the higher seropositivity among dairy farm workers[4]. Among slaughterer house workers Brucella seropositivity was found (0.0%), meat shop workers (10.5%) and veterinarians (8.3%). Study from Pakistan conducted by Masoumi[21] revealed that (8.33%) seropositivity among slaughterhouse workers. Relevant study conducted by Nikokar et al[22] from Iran reported (9.8%) seropositivity. It was higher than the study carried out in Pakistan. We knew that, during slaughtering activities slaughterhouse workers were directly exposed to viscera and blood of infected animals. These were associated with a higher risk of brucellosis[23]. From this study we found no IgM seropositivity among slaughterhouse workers. IgM seropositivity is generally found in acute infection cases. Most of the workers in slaughterhouses were performing their jobs for more than 20 years explaining the absence of IgM in their blood[24]. Mukhtar and Kokab[25] from Pakistan examined n=166 serum sample from meat-shop workers and showed 30(18.1%) seropositivity that was higher than this study. Chowdhury[24] from Bangladesh and Aworh[26] from Nigeria both of their studies established that abraded skin or cuts on bare hand as a route of brucella infection for most abattoir workers.

The present study showed that the brucellosis is still a professional hazard in the veterinary practitioners. Studies from Bangladesh conducted by Rahman et al[27] reported (5.3%, n=19) seropositivity in veterinary personnel. These finding were lower than this study. A higher rate of prevalence in veterinarians was reported by Kumar et al[28] (28.57%). Veterinarians are repeatedly handled diseased animals and parturient animal. They also practiced artificial insemination. For that reason, they are getting infected by Brucella. In this study the high-risk professionals were related with cattle or goat handling. Some of them handled both.

We found that 16 participants handled both cow and goat, among whom 9(56.3%) were IgG seropositive. Seropositivity was found in 54.1% and 25.0% among the cattle and goat handler respectively. It was also observed that brucella seropositivity and type of animal handled showed no significant outcome. (Chi-square =3.479; P>0.05). A Bangladeshi study conducted by Rahman et al[27] reported the highest seropositivity was found (59.8%) among the goat handler followed by 4.5% seropositivity in people who handled both cattle and goat. In the same study they found that 14.2% livestock farmers shared same premises with animals. Diseased animals can excrete Brucella through urine, in this way the bacteria are disseminated and infect other animals and humans[29]. In this study we observed that veterinarians, as a part of their professional duty always had to handle both the diseased cattle and goats. In this study the data showed that consumption of raw milk was one of the most important risk factors among the participants (n=20, 30.8%). The IgG seropositivity in 11(55%) cases among these 20 raw milk consumers. A separate study conducted by Hernandez[20] from Mexico, 24.1%, n=58 seropositivity was found in participants having history of unpasteurized dairy consumption[0]. Which is not in accordance with this study finding. In this study more seropositivity observed may be due to easy access of the participants to raw milk due to their profession. Moreover, in some cases these people were lacking in the knowledge of food hygiene or disease transmission risk from raw milk consumption.

Other study published by Sofian[8] from Iran has also reported the habitual intake of raw milk as the probable cause of brucellosis. Brucellosis is a clinical condition with some signs and symptoms by which a provisional diagnosis can be made by the physicians. We asked all the participants about any clinical complaints. Among them, (33.84%, n=22) participants were presented with the symptoms commonly seen in brucellosis like irregular fever, joint pain during data collection. IgG antibodies were measured among symptomatic participants (n=22) and seropositivity was found in 13 (59.1%) cases. A seroprevalence study on Brucellosis conducted by Rahman[27] from Bangladesh showed (28.0%) seropositivity in symptomatic individuals (n=60). This finding was lower than this study finding.

Some significant facts about the living style, personal hygiene, immunity among the high-risk professional participants emerged from this study. In this study the IgG seropositivity was mostly occurred in participants with middle class socioeconomic condition (62.5%). We found that middle class participants were veterinarians by occupation and that is why they were constantly in close contact with the source of brucella. The overall Brucella IgG seropositivity was found in 32 (49.2%) cases. From this study it can be ascertained that among those in high-risk participants infection was mainly due to contact with animals and their products and raw milk consumption.

Conclusion

This study reflects that the very serious extent of human brucellosis in the presently investigated population. The study is a small one involving limited number of participants in a defined geographical region. But considering the relative evenness of the population throughout the country practicing similar behavioral pattern the result can safely be extrapolated to whole geographical region of Bangladesh. Brucella seropositivity was found in high-risk occupational population. Animal contact seems to be the main mode of transmission. Brucella Vaccination of domestic livestock should be included in their vaccination schedule to prevent continuous transmission of brucella and occurrence of human brucellosis. Protective measures for high-risk professionals should be taken by using protective clothing, gloves, masks and goggles to prevent occupational related brucellosis. A control program for human brucellosis would depend to a large extent on public health education about the disease and its risk factors, food hygiene, personal hygiene and good administrative arrangement. Active co-operation between health services and veterinary services should be promoted.

Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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References

1. Murray PR and Corbel MJ. Brucella. In: Borellio SP, Murray PR, Fanke G, editors. Topley and Wilson Microbiology and Microbial Infections, 10th ed. PRESS; 2007: pp. 1719-51.
2. Mantur BG and Amarnath SK. Brucellosis in India – a review. J Bio sci 2008; 33: 539-7.
3. Corbel MJ. Brucellosis in humans and animals. WHO, 2006
4. Sammartino LE, Gil A, Elzer P. Capacity building for surveillance and control of bovine and caprine brucellosis. ExpConsult Com Vet Pub H Sci 2004; 3: 55-66
5. Malik GM. A clinical study of brucellosis in adults in the Asir region of southern Saudi Arabia. Am J Trop Med Hyg 1997; 56: 375-7.
6. Scholz, H.C.; Hubalek, Z.; Sedlacek, I.; Vergnaud, G.; Tomaso, H.; Dahouk, S.A.B. Microti sp. Nov., isolated from the common vole Microtus arvalis. Int. J. Syst. Evol. Microbiol. 2008, 58, 375–382.
7. Rahman MS, Uddin MJ, Park JH, Chae JS, Rahman MB, and Islam MA. A short history of Brucellosis: special emphasis in Bangladesh. Bangl J Vet Med 2006; 4: 01–06.
8. Sofian M, Aghakhani A, Velayati AA, Banifazl M, Eslamifar A, Ramezani A. Risk factors for human brucellosis in Iran: a case-control study. Int J Infect Dis 2008; 12: 157-61.
9. Prakash P, Bhansali S, Gupta E, Kothari D, Mathur A, Ambuwani S. Epidemiology of Brucellosis in high risk group and PUO patients of Western – Rajasthan. N J Com Med 2012; 3: 61-65.
10. Pappas G, Papadimitriou P, A kritidis N, Christou L, Sianos EVT. The new global map of human brucellosis. Lancet Infect Dis 2006; 6: 91-9.
11. Khorvash F, Keshteli AH, Behjati M, Salehi M, Naeini AE. An unusual presentation of brucellosis, involving multiple organ system, with low agglutinating titers: a case report. Journal of Medical Case Reports 2007; 1(53): 1-3.
12. Dean AS, CrumpL, Greter H, Hattendorf J, Schelling E, Zinsstag J. Clinical menifestations of human brucellosis: A systemic review and meta-analysis. PLOS Neg Trop Dis 2012; 6: 1-8.
13. Ahmed BN, Islam ABM, Sultan Y, Nahar K, Hassan KM, Jamil KM, & Alamgir AKM. Brucella antibodies titers among cattle handlers in rural community of Bangladesh. Bangla J pathol 2006; 21: 20-24.
14. Nikokar I, Hosseinpour M, Asmar M. Seroprevalence of brucellosis among high risk individuals in Guilan, Iran. J Res Med Sci 2011; 16: 1366–71.
15. Rahman MM, Chowdhury TIMFR, Rahman A, et al. Seroprevalence of human and animal brucellosis in Bangladesh. Indian Vet J 1983;60:165-168.
16. Renukaradhya, G.J.; Isloor, S.; Rajasekhar, M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet. Microbiol. 2002, 90, 183–195.
17. Agasthya AS, Isoor S, and Prabhudas K. Brucellosis in high risk group individuals. Ind J Med Microbiol 2007; 25: 28-31.
18. Rahman Md S, Sarker RR, Melzer F, Sprague LD, and Neubauer H. Brucellosis in human and domestic animals in Bangladesh: A review. Afr J microbiol Res 2014; 8: 3580-94.
19. Muhammad N, Hossain MA, Musa AK, Mahmud MC, Paul SK, Rahman MA, Haque N, Islam MT, Parvin US, Khan SI, Nasreen SA, Mahmud NU. Seroprevalence of human brucellosis among the population at risk in rural area. Mymen Med J 2010; 19:1–4.
20. Hernandez MEC, Vazquez AO, Gonzalez PT, Mazari BC, Ramos OS, Osornio JS, Leon APD, Valle MBD. Seroprevalence of brucellosis among dairy farm workers in Mexico. Salud Pub Mexico 2016; 58: 366-70.
21. Masoumi J P, Sheikh M A, Ahmad R, Naeem M, Ahmad M, Hussain I. Seroprevalence of Brucellosis in sheep, goats and man in Lahore area. Ind J of Dairy Sci 1992;14:298–9.
22. Nikokar I, Hosseinpour M, Asmar M, Pirmohbatei S, Hakeimei F, Razavei MT. Seroprevalence of Brucellosis among high risk invividuals in Guilan, Iran. J Res Med sci 2011; 16(10): 1366-71.
23. Acharya D, Hwang SD, Park JH. Seroreactivity and Risk Factors Associated with Human Brucellosis among Cattle Slaughterhouse Workers in South Korea. Int J Environ Res. Public Health 2018; 15(2396): 1-9.
24. Aworh MK, Okolocha E, Kwaga J, Fasina F, Lazarus D, Suleman I, Poggensee G, Nguku p, Nsubuga p.Human brucellosis: seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria-2011. Pan Afr Med J 2013;16:103-8.
25. Mukhtar F and Kokab F. Brucella serology in Abattoir workers. J Ayub Med Coll Abbottabad 2008; 20: 57-61.
26. Chowdhury OA, Ahmed F, Ahbab MA. Serosurvey of Brucella Antibody in Abattoir worker. Syl Med J 2000; 21: 11-16.
27. Rahman AKM A, Dirk B, Fretin D, Saegerman C, Ahmed MU, Muhammad N, Hossain A, Abatih E. Seroprevalence and risk factors for Brucellosis in high risk group of individual in Bangladesh. Food path dis 2012; 9: 190-97.
28. Kumar P, Singh DK, Barbuddhe SB. Sero-prevalence of brucellosis among abattoir personnel of Delhi. J Commun Dis 1997;29:131–7.
29. Doganay M and Aygen B. Human brucellosis: an overview. Int J Infect Dis 2003; 7: 173 82.