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ORIGINAL ARTICLE

Potential Risk Factors Analysis of Dairy Cattle Management Against Brucellosis

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Abstract

The objectives of this study were to determine the serological prevalence of brucellosis in crossbred dairy cattle on the selected dairy farms and to identify management related risk factors associated with the malady. The number of positive reactors by RBPT was 14 out of 334 and the overall animal-level serological prevalence of brucellosis within the herd was 4 out of 334 confirmed by I-ELISA. The prevalence was relatively higher in females than that in males. A significant association was found between abortion and age of the animals, and the occurrence of the disease. Results of this study revealed that several farm factors can be considered as potential risk factors which increase the risk of an animal being infected with *Brucella* infections. Among these are the housing type ($p=0.059$, OR=3.36), certain variables of farm security such as the recording of visitors ($p<0.001$, OR= 18.06), and an awareness of the disease ($p<0.001$, OR=18.47). The presence of visitor's record shows a statistically higher risk of getting the disease than farms that do not keep records. In addition, workers who had never heard before about the disease were more threatened than those having at least a basic knowledge of the disease. As the brucellosis does affect people and livestock of Bangladesh, therefore there was an immediate need for a concerted effort to control and eradicate brucellosis from domesticated animals in Bangladesh.

Key words: Brucellosis, Prevalence, Risk factors, Dairy cattle.

Introduction

Worldwide, brucellosis remains an important disease in humans, domestic and wild animals (OIE, 2009). It is an infectious disease caused by bacteria of the genus *Brucella* which comprises eight species ranked according to their pathogenicity and host preferences. Six of the eight species can be isolated from terrestrial mammals: *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis et B. neotomae* (Halling and Young, 1994). Indeed, brucellosis has an important impact on the health and productivity of livestock greatly reducing their economic value (Ly, 2007). The epidemiology of brucellosis is complex and the prevalence varies across geographic regions and livestock systems. The disease incidence is influenced by management factors, herd size, population density, type of animal breed and biological features such as herd immunity (McDermott and Arimi, 2002; Acha and Szyfres, 2005; Muma *et al.*, 2007; Mekonnen *et al.*, 2010; Makita *et al.*, 2011; Megersa *et al.*, 2011).

Transmission of *Brucella* to humans results from direct contact with the infected animal, consumption of unpasteurized milk and milk products (Corbel, 2006; Mantur *et al.*, 2006). Human brucellosis is mainly an occupational disease affecting animal caretakers, livestock farmers, artificial inseminators, abattoir workers, meat inspectors and veterinarians due to frequent exposure to infected animals (Corbel, 2006). Close contact with animals may occur when humans assist animals during parturition or abortion or handling of stillbirth. Inhalation of *Brucella* has been reported in slaughterhouse workers where the concentration of organisms can be high due to aerosol generation (Sammartino *et al.*, 2005). Dairy farmers who milk animals by hand have a greater chance of

becoming infected from the *Brucella* infected animals (Sammartino *et al.,* 2005). Transmission of brucellosis in animal results from ingestion of contaminated feeds and water, inhalation of aerosolized bacteria, sexual intercourse and direct contact with infected placenta and uterine discharges (Corbel, 2006; Radostits *et al.,* 2007). Vertical transmission of *Brucella* is also reported from infected cattle or dam to calf, lambs or kids. There are two main factors associated with an animal's susceptibility to *Brucella* infection. First, brucellosis primarily affects sexually mature animals (Havas, 2011; Sammartino *et al.,* 2005). Second, susceptibility dramatically increases during pregnancy (Havas, 2011; Sammartino *et al.,* 2005). Understanding the mode of transmission of *Brucella* is important because it plays a key role in the disease epidemiology. Major risk factors of animal infection are the husbandry practices, local habits and management of the herd/flock. The size of the herd, housing methods and population density are factors that have been linked to the progression of the disease. The aim of this study was to determine the serological prevalence of *Brucella* infection in crossbred dairy cattle on the selected farms and to identify risk factors for infection both in human and livestock populations.

Materials and Methods

This study involves a questionnaire based survey of farmers as well as blood sampling from their animals. Participants provided their verbal informed consent for animal blood sampling as well for the related survey questions, according to the procedures at the time of the study. Collection of blood samples was carried out by professional veterinarians adhering to the regulations and guidelines on animal husbandry. Farmers were not forced to participate in the survey and animal blood sampling. Paper questionnaires were encoded and recorded in Excel and names were replaced by their coded versions for analysis.

Study Area

The Mohammadpur beribadh is located in the north-west corner of Dhaka district and lies between latitude $23^0 46'$ N and longitude $90^0 23'$ E. The study was carried out in the cross-bred dairy cattle farms of Mohammadpur beribadh area, Dhaka, Bangladesh.

Questionnaire Design and Data Collection

The study took place between October 2010 and March 2011. Information about each herd and the animals kept was collected by means of a structured questionnaire, which was completed at all the selected herds on a single visit. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses. Important herd and animal level data recorded included cattle location, source of forages (home established, road side, or bought-in hay), sex, breeding method used (natural or artificial insemination), source of drinking water (tap, rain water, shallow well, river, pond), contact with other animals and place (between farms, during grazing, at water source, during mating), herd owner education level (binary variable: illiterate and primary education), and the number of years in livestock farming. Other information sought included history of abortion and vaccination, reproductive diseases, disposal of afterbirth, age determined from birth records and dentition characteristics, and the type of floor in the animal house (concrete, dirt) as well as whether or not a system of grazing or zero-grazing was practiced. The study also recorded some clinical, epidemiological and reproductive information (Table 1).

Blood was collected from the jugular veins into Vacutainer® tubes, which were immediately placed into an ice bath and transported to the laboratory within a maximum of 7 hours. When the outside ambient temperature was cool, the clot was allowed to form in the Vacutainer® tube in the field before transportation. The samples were centrifuged at 3,000 rpm for 15 minutes and the serum was removed and stored at -20°C until analyzed. The collected samples were then used for laboratory analysis using the Rose-Bengal plate-agglutination test (RBPT) and the positives were confirmed by an indirect enzyme-linked immunosorbent assay (I-ELISA). The laboratory tests were performed in Bangladesh Livestock Research Institute (BLRI) laboratory, Savar, Dhaka.

Data Management and Statistical analysis

The questionnaire data was transferred into a Microsoft Excel spreadsheet. Database errors were rechecked also by using the Microsoft Excel program. With regard to the study objectives of this research, the statistical analysis and calculation of specific sample prevalence data of brucellosis at a particular level are performed by using the following:

- The data collected from questionnaire survey were analyzed using descriptive statistics.
- Win Episcope 2.0 program was used for the calculation of the sensitivity, specificity, the predicted positive and negative values of the RBPT test and the I-ELISA test.
- Association between a potential risk factors and proportion of disease free individuals and infected individuals was expressed by the Odds Ratio (OR) with 95% confidence interval (CI).

Table 1: The summary of the Questionnaires and checklist

Risk Factors	Checklist	Description
General Characteristics	Owner	Name, address, telephone no., sex, age, educational level, farming experience
	Origin of Farm	Name and location, farm size, breed, age, sex, source of animals, identification number or particular name of animal
Introduction of infected animals into the herd	Disease monitoring system	Vaccination program, breeding program, milking and production systems
Level of management system of in the farm	Management system	Type of operation, housing, feeding
	Recording system	Animal record files, herd production files, herd disease files, herd environment or management files
Exposure of the herd to infectious disease	Biosecurity	Unauthorized visitors control, animal movement control, contact with other species and livestock, personnel hygiene, farm sanitation, cleaning and disinfection, quarantine
	Health problem	Veterinary service, clinical signs present or experienced during last year
Exposure of the herd to infectious disease	Biosecurity	Unauthorized visitors control, animal movement control, contact with other species and livestock, personnel hygiene, farm sanitation, cleaning and disinfection, quarantine
	Health problem	Veterinary service, clinical signs present or experienced during last year

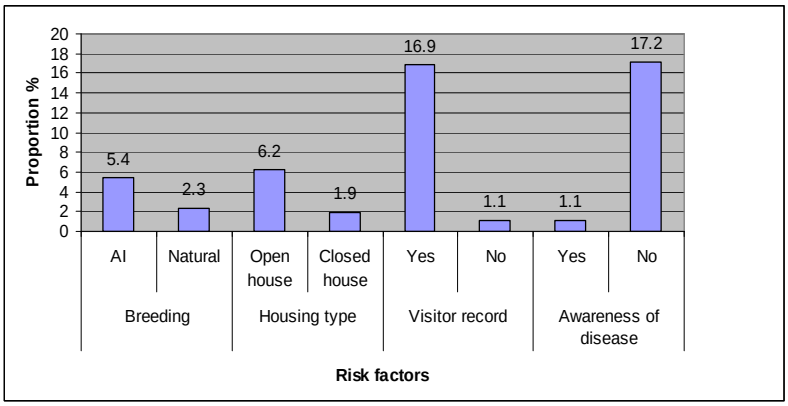

Fig 1: The proportions of *Brucella* infected animals by potential risk factors.

Results

The number of positive reactors by RBPT was 4.20% (14/334) whereas the overall animal-level sero-prevalence of brucellosis within the herd was 1.20% (4/334) confirmed by I-ELISA. In this study, we found the higher prevalence rate of brucellosis in female cattle than males. The prevalence was also found to be high in older ages (> 4 years) and among those that had the history of previous abortions (data not shown). Finally, the objective of the questionnaire survey was to determine the potential risk factors of the management practices of the disease free and infected individuals. The results deal with the distribution of selected possible risk factors related to *Brucella* infected and non infected individuals. Among the management factors, the type of operation was fully

intensive (100%). Most animals (53.3%) of the housing type were being reared in the open house, while others (46.7%) were keeping in the closed housed. However, (33.2%) of the houses had a concrete floor while (66.8%) had un-concrete floor. All animals were being fed through manual feeding systems (100%).

The potential risk factors which may be associated with *Brucella* infection are presented in Table 2. The analysis of risk factors did not reveal significant impact on the development of *Brucella* infection in the animals. On the contrary, there was no interaction of farm management characteristics between particular management system variables. However, there was no statistically significant difference on the issue of the housing system: the p-value of 0.059 which was very close to the significant level ($p \leq 0.05$) may be explained by a fact the open house system had a higher percentage (6.2%) of infections than the closed house system (1.9%) owned by higher risk of OR=3.36.

Table 2: Analysis of potential risk factors of introduction of infection related to *Brucella* infected individuals

Variables	No. of individuals	No. of positive individual	Positive (%)	95% CI* (Lower & Up limit)	Fisher's Exact test P value (α=0.05)	OR* (95% CI)
Introduction of infection						
Breeding						
Artificial Insemination	202	11	5.4	[2.89, 9.79]	0.263	2.48
Natural	132	3	2.3	[0.59, 7.01]		[0.68, 9.05]
Milking						
Hand milking	1	0	0	[0.0, 48.5]	1.00	
Machine milking	333	14	4.2	[2.41, 7.12]		
Production system						
Dairy	334	14	4.2	[2.41, 7.12]	1.00	
Others	0	0	0	[0.0, 0.0]		
Total	334	14				

**Confidence Interval, *Odds Ratio*

Table 3: Analysis of potential risk factors on exposure of disease related to *Brucella* infected individuals

Variables	No. of individuals	No. of positive individual	Positive (%)	95% CI* (Lower & Upper limit)	Fisher's Exact test P value (α=0.05)	OR* (95% CI)
Exposure of disease						
Control visitors						
Yes	47	4	8.5	[2.76, 21.27]	0.118	2.58
No	287	10	3.5	[1.78, 6.51]		[0.77, 8.58]
Visitors record						
Yes	65	3	1.1	[0.29, 3.50]	0	18.06
No	269	11	16.9	[9.15, 28.69]		[4.87, 66.92]
Vehicles disinfection						
Yes	26	2	7.7	[1.34, 26.60]	0.298	2.06
No	308	12	3.9	[2.12, 6.89]		[0.43, 9.72]
Quarantine practice						
Yes	109	4	3.7	[1.18, 9.68]	1	0.82
No	225	10	4.4	[2.27, 8.26]		[0.25, 2.67]
Veterinary services						
Yes	332	14	4.2	[2.41, 7.14]	1	_
No	2	0	0	[0.0, 48.5]		
Awareness of the disease before						
Yes	270	3	1.1	[0.29, 3.48]	0	18.47
No	64	11	17.2	[9.29, 29.10]		[4.98, 68.47]
Total	334	14				

**Confidence Interval, *Odds Ratio*

The potential factors carrying the risk of introducing the infection have been analyzed in Table 3. The analysis results in Table 3 show two important risk factors- the visitor's records and awareness about potential disease. The farms which did not record the presence of visitors showed 16.9% more chance to get infection as compared to 1.1% where the farms were keeping such records. There was statistically higher risk of getting the disease at farms that did not keep records ($p<0.001$, OR=18.06). In addition, workers (17.2%) who had never heard about the disease were more at risk than workers (1.1%) who were having a basic knowledge of the disease, which was significant at $p<0.001$ and OR=18.47.

Discussion

Brucellosis is an important zoonosis and serological surveillance is essential to its control (Erdenebaatar *et al.,* 2004; Raghunatha Reddy *et al.,* 2014). Although the eradication programs have been established by vaccination, test and slaughter of the *Brucella* infected animals, but the disease still remains as a major zoonosis all over the world (Matyas and Fujikura, 1984; WHO, 1986; Baek *et al.,* 2003; Kakoma *et al.,* 2003; Madhavaprasad *et al.,* 2014) and is prevalent in many countries. The results of the present study on the basis of questionnaire and from the direct observations of all the animals, it is clear that several factors can be considered as potential risk factors which may increase the risk of an animal picking up *Brucella abortus* infection. Among these are the housing type ($p=0.059$, OR=3.36), certain variables of farm security such as keeping the visitors records ($p<0.001$, OR= 18.06), and an awareness of the disease ($p<0.001$, OR=18.47). These findings were in agreement with those of Salman and Meyer (1987) who observed that the risk factors which influence the spread of bovine brucellosis were related to the animal population, management, and the biology of the disease. The variables which contribute significantly to seropositive animals are the size of the farm, the percentage of animals that are inseminated artificially, the size of the investment in livestock, and the policy of the owner with regard to the disposal of the reactor animals. Some workers (Crawford, 1990; Enright, 1990) have reported that the risk factors associated with the spread of the disease within a herd include unvaccinated animals in the infected herds, herd size, population density, and type of housing and the use of maternity pens.

As far the results concerned with the introduction of the infection into the animals by vaccination, it is clear from the response that none of the animals were vaccinated against brucellosis. Among the respondents, 334 (100%) animals were vaccinated against Foot and Mouth Disease (FMD), Haemorrhagic Septicemia (HS), Anthrax, and Black Quarter (BQ). It needs to be mentioned that 132 (39.5%) animals were being naturally bred with the help of their bulls (natural insemination). On 202 (60.5%) animals, artificial insemination was being carried out which showed higher percentage of positive cases. Regarding the milking procedure, 99.7% were collecting the milk by milking machine. The production systems commonly found in the study area were of dairy type (334 or 100%). While 19.2% of respondents had not ever heard of brucellosis Before but 80.8% of the 334 respondents were having fundamental knowledge on the disease. Open type houses were associated with the presence of *Brucella* antibodies, although this was not statistically significant ($p=0.06$) but showed higher risk at OR=3.36. Open houses are likely to allow frequent contact between animals, birds or vectors. The same applies for the slightly increased prevalence in free ranging farms as compared to intensive farms. Interestingly, factors that are expected to be protective like control of visitors, the adoption of this control measure may have been a consequence of experience with the disease. The results have shown that the higher risk of getting the disease was through contact with visitors. There are also reports that the use of artificial insemination was not significantly protective against *Brucella* infections as suggested by Salman and Meyer (1987). The use of a disinfectant to clean the vehicles visiting the farms for several reasons took place in 7.8% of all cases, whereas in 92.2% of all cases it did not. As regard veterinary services, the majority of respondents said that these were available (99.4%) and sometimes unavailable (0.6%). With regard to checklist of risk factors, the proportions of significant positive results are differentiated by means of introduction of infection, the management system and the exposure of disease.

The differences in seropositive reactors between male and female indicate that the risk of infection with *Brucella* spp. is higher in female animals. The variation of prevalence of brucellosis between animals on farms and domestic holding are likely to be attributed to certain risk factors such as cattle management practices, population dynamics and biological features (e.g. host immunity) that largely influence the epidemiology of *Brucella* spp. (Al-Majali *et al.,* 2009; McDermott and Arimi, 2002; Reviriego *et al.,* 2000). The preponderance of seropositive reactors in mature and pregnant animals suggests that sexually mature and pregnant animals are at higher risk of infection with *Brucella* spp. (Muma *et al.,* 2007). Higher prevalence

of brucellosis in animals with a history of abortion constitutes a significant risk for transmission of brucellosis to the uninfected animals since they are known to shed massive number of *Brucella* from the uterus at subsequent normal parturitions.

The prevalence and severity of the infection may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factors (Amin *et al.,* 2005). Therefore, a relatively lower sero-prevalence observed in this study may be due the selected farms, smaller sample size and also to the applied diagnostic methods. The present study mostly included the cross-bred dairy cattle from five selected farms which follows a semi-intensive health management system. The serosurveillance studies of brucellosis in humans and animals suggest that brucellosis is endemic in Bangladesh (Rahman, 1983; Muhammad *et al.,* 2010; Rahman *et al.,* 2012a; Nahar and Ahmed, 2009; Ahasan *et al.,* 2010; Rahman *et al.,* 2012b; Sikder *et al.,* 2012). Without control measures, the infected animals will continue to serve as reservoirs for the spread of the disease to uninfected animals and humans. As has been shown in other countries, controlling the disease in animals contributes significantly to the decreased incidence in humans (Jiang and Baldwin, 1993). While the overall ser-prevalence is relatively low (1.20%), brucellosis is an important threat to the health of animals and humans. Therefore, the results obtained from this study can provide valuable information regarding the epidemiology of brucellosis in farmed cattle in Bangladesh as well for controlling the infections of this zoonotic malady.

Conclusion

Brucellosis is a serious problem among cattle worldwide. However, to encourage voluntary farmer participation and to raise awareness about the risks related to the disease in animals and human beings, an extensive public awareness campaign is vital, as well as strict and mandatory dairy movement control. The variation of prevalence of brucellosis between animals on farms and domestic holding are likely to be attributed to certain risk factors such as cattle management practices, population dynamics and biological features (e.g. host immunity) that largely influence the epidemiology of *Brucella* spp. Public health education for target groups of people, understanding the risk factors of brucellosis, hygienic animal management practice (bio-safety), early diagnosis, collaboration among veterinarians, medical doctors and farmers and vaccination of animals are necessary for control of brucellosis.

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