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RESEARCH ARTICLE

# Bovine Brucellosis: An Epidemiological Study at Chittagong, Bangladesh

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**ABSTRACT**

An epidemiological survey was conducted to identify probable risk factors and prevalence of brucellosis in commercial and backyard dairy cows at Chittagong, Bangladesh. A total of 500 milk samples were collected (250 commercial and 250 backyards) for Milk Ring Test (MRT). The MRT positive cows were subjected to sera collection and Rose Bengal Plate Test (RBPT) and indirect ELISA were done for confirmatory diagnosis. The overall seroprevalence of brucellosis in cattle was 5% (7.6% in commercial and 2.4% in backyard). Significantly higher ($P<0.05$) prevalence was found in the zero grazing (5.74%), pregnant cows (7.53%) and cows with history of retained placenta (7.89%) or abortion (5.88%) or both (11.76%) than non-pregnant (2.68%) and without any reproductive disorder (4.44%). A total of 420 farm attendants and owners were interviewed where 93.55 and 99.08% commercial and backyard personnel were found to have no knowledge of brucellosis and 9.67 and 87.77% consumed raw milk and yogurt respectively were highly vulnerable to zoonotic brucellosis. The results showed that brucellosis is widely distributed locally, underscoring the need for further studies including biovar determination.



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## INTRODUCTION

Brucellosis is considered to be the most widespread bacterial zoonotic disease worldwide. *Brucella abortus* is the principal cause of brucellosis in cattle (Radostits *et al.*, 2000; Abubakar *et al.*, 2012). Brucellosis is essentially a disease of sexually matured animals. It mainly affects reproduction and fertility, reduces the survival rate of newborns and milk yield. There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which are thought to be due to brucellosis (Munir *et al.*, 2010; Maadi *et al*., 2011).

Milk Ring Test (MRT), Rose Bengal Plate Test (RBPT) and ELISA are generally used for the detection of *Brucella* infection in livestock. Sero-prevalence of bovine brucellosis in Asia can be summarized as: 5% in India (Renukaradhya *et al.*, 2002), 3% in Pakistan (Shafee *et al*., 2011) and 4.7% in Sri Lanka (Silva *et al.*, 2000). In Bangladesh, Rahman *et al*. (2006) reported the seroprevalence of bovine brucellosis as 2.4-18.4%, while the herd seroprevalence was 62.5%. Amin *et al.* (2005) reported higher prevalence in rural farm cows (5%) than organized farm cows (2.5%). But there is no Chittagong based study which is considered as the milk pocket of the south-east part of Bangladesh. Therefore, the presented study was carried out to estimate the prevalence and associated risk factors of brucellosis in cows of both commercial and backyard dairy farming system.

## MATERIALS AND METHODS

**Study area and population:** The study was conducted only on the lactating dairy cows in Chittagong district, Bangladesh from February to July, 2011. Stratified random sampling method was followed to select the cows. A total of 250 cows were selected from 7 commercial

farms (5 farms from city and 2 from rural areas and each having 14-63 cows). Similarly, 250 cows were selected from 140 backyard farms (considering a farm that kept at least a lactating cow). The backyard cows were chosen from 5 different upazillas namely Hathazari, Shitaqund, Potia, Anwara and Banskhali (50 cows from each upazilla). The type of cow kept under commercial farming system was Friesian (*Bos tourus*) cross of indigenous (*Bos indicus*) and in the backyard system mostly of indigenous type.

**Questionnaire design and data collection:** Information about each herd, cows reared and attendants was collected by means of a structured questionnaire. The questionnaire was designed to mostly close ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses (Thrusfield, 2005). The questionnaire was filled up by interviewing the farmers, farm manager, attendants and taking records from registers wherever necessary. Important herd level data collected were location, type and size of farm, history of vaccination and disposal of afterbirth. Significant animal level data recorded were breed, age (determined from birth records), history of vaccination and breeding method (natural or artificial) as well as whether or not a system of grazing was practiced. Pregnancy and lactation status, history of abortion, retained placenta or other reproductive disorders was also collected. Herd personnel's knowledge on brucellosis, use of protective hand gloves during handling of aborted materials and history of raw milk consumption were also recorded from zoonotic point of view.

**Sample collection and processing:** Approximately 5ml of milk was collected from four quarters (after disinfection of udder with potassium-per-manganate solution) of each cow into sterile screw capped vial (Becton Dickson, UK) and stored in the ice box. Within 6 hours of collection the samples were screened by Milk Ring Test (MRT) as described by Shafee *et al.* (2011). The cows that showed positivity to MRT were subjected to blood collection (within 2 days of MRT) for separation of sera which stored in 1.5 ml eppendorf tubes at -20°C until serological tests were performed.

Antibodies to *Brucella* spp. were detected by sequential testing of samples using the indirect ELISA (iELISA kit, M/S Svanova Biotech AB, art. No. 10-2700-10, SE-751 83 Uppsala, Sweden) and RBPT for confirmation. The RBPT antigen was supplied by VLA Weybridge, UK. The test procedure recommended by Alton *et al.* (1988) was followed. A cow was considered to be positive if it tested positive on all three tests: the MRT, iELISA and RBPT.

**Data analysis:** Data from the questionnaires and laboratory results were stored in personal computer, using Microsoft Excel spreadsheet program 2007. Descriptive statistical analyses of various risk factors and dependent variables were done using Intercooled STATA 9.0 (Stata Corporation 2008). Proportional analysis and logistic regression was used to interpret the data.

## RESULTS

**Participating farms characteristics:** A 100% response was found when selected farms were visited and the farmers were interviewed. All the commercial and 17.14% (24/140) backyard farms practiced different vaccinations (anthrax, BQ, HS and FMD) but there was no history of *Brucella abortus* strain 19 vaccination among the farms recorded. No maternity pen was maintained by the backyard farms, while 28.57% (2/7) commercial farms upheld it. In a hostile way 76.43 (107/140) and 28.57% (2/7) backyard and commercial farms respectively disposed aborted materials by throwing elsewhere.

**Prevalence and risk factors analysis:** The sero-survey results are presented in the Table 1. Of the 500 sampled cows, serological results were available from 88 cows as the cows that showed negativity to Milk Ring test were considered as negative to brucellosis. The association of the animal explanatory variables and brucellosis is shown in Table 2. The categorical variables that qualified at $P<0.05$ during univariable analysis were grazing, replacement, pregnancy and reproductive disorders.

**Table 1:** Overall and comparative prevalence (commercial and backyard) of brucellosis

| | Total Sample | Test Positive | Prevalence (%) | 95% CI |
|---|---|---|---|---|
| Overall | 500 | 25 | 5.0 | 3.08-6.92 |
| Commercial | 250 | 19 | 7.6 | 4.29-10.91 |
| Backyard | 250 | 6 | 2.4 | 0.49-4.31 |

**Table 2:** Association of brucellosis and explanatory variables in logistic regression model

| Variables | | Prevalence % | 95% CI | P value |
|---|---|---|---|---|
| Breed | Local | 0.85 | -0.83-2.53 | >0.05 |
| | Cross | 6.28 | 3.84-8.73 | |
| Parity | 1,2 | 4.78 | 2.23-7.33 | |
| | 3,4 | 4.03 | 0.84-7.22 | |
| | >4 | 7.59 | 1.63-13.57 | |
| Pregnancy | Yes | 7.53 | 4.16-10.91 | <0.05 |
| | No | 2.68 | 0.71-4.65 | |
| Reproductive disorder | None | 4.44 | 2.48-6.39 | |
| | Abortion | 5.88 | -6.58-18.35 | |
| | Retained placenta | 7.89 | -1.08-16.88 | |
| | Both | 11.76 | -5.31-28.84 | |

**Participating farm owners and attendants observations:** A total of 420 cattle attendants and farm owners (93 commercial and 327 backyards) were interviewed to investigate the risk factors from zoonotic aspect. It was observed that 93.55 (87/93) and 99.08% (324/327) personnel had no knowledge of brucellosis, respectively. No history of wearing protective gloves was recorded in backyard farm attendants, while 79.57% (74/93) commercial farm attendants used it during handling of aborted materials. Furthermore, 87.77 (287/327) and 9.67% (9/93) backyard and commercial attendants responded positively on the consumption of raw milk as yoghurt.

## DISCUSSION

The overall prevalence of brucellosis in cattle was comparable with Rahman *et al.* (2006), Renukaradhya *et al.* (2002) and Swai and Schoonman (2010) which

reflected a past or present exposure to *Brucella* organisms because vaccination against brucellosis has never been practiced in Bangladesh. However, Amin *et al.* (2004), Shafee *et al.* (2011) and Tesfaye *et al.* (2011) found lower prevalence rate than the present study. On the other hand, Abbas and Aldeewan (2009) and Ahmed *et al.* (2010) reported higher rate of prevalence. The apparent geographical variation in the seroprevalence might reflect differences in the levels of natural immunity. In addition, sensitivities and specificities of the diagnostic methods used among researchers might also influence the outcome.

The prevalence rate of commercial dairy cow was almost correlated with the observation of Munir *et al.* (2011), who recorded 6.5% by RBPT. However, Amin *et al.* (2005) reported lower prevalence rate in commercial dairy cow. On the other hand, prevalence rate of backyard cows was in consistent with the findings of Berhe *et al.* (2007) and Omer *et al.* (2000a). Nevertheless, Makita *et al.* (2011) found higher prevalence in backyard cows than the present study. Variation in management and husbandry practices employed and disease resistance among the breeds maintained in the systems might be accountable for difference in prevalence rate.

The study also revealed that the prevalence was higher in cross bred cow than local or indigenous cows which were approved by Omer *et al.* (2000b). The higher prevalence in cross breds might be due to malnutrition, poor husbandry practices and tropical environmental stress.

In addition, prevalence in pregnant cows was near to the finding of Amin *et al.* (2005), whereas Nahar and Ahmed (2009) set up higher rate of prevalence than the present study. Moreover, prevalence in non-pregnant cows was comparable with the findings of Nahar and Ahmed (2009). Infected reproductive tract of cows could act as a potential reservoir for the organisms to propagate and become active during pregnancy which might be the cause behind higher prevalence rate in pregnant cows.

Mutiparous cows showed increased prevalence to brucellosis which was supported by Matope *et al.* (2011) although the result was not statistically significant. Moreover, history of abortion or retained placenta was found as risk factors associated with brucellosis which was endorsed by Berhe *et al.* (2007).

The questionnaire survey of 420 cattle attendants and owners in the farms showed that large number of them have no knowledge of brucellosis. This might be due to poor training facilities among the farmers and farm attendants. Furthermore, percentage of commercial farm attendants in practicing protective gloves was higher than the findings of Tesfaye *et al.* (2011). Presence of high association between brucellosis and abortion as well as retained placenta was indicative of risk to cattle attendants and professionals working in the area without precautions and protective clothes (Radostits *et al.*, 2000).

Additionally, 9.67 and 87.77% commercial and backyard personnel responded positively to the consumption of raw milk as yoghurt respectively which integrated the finding of Sabah *et al.* (2008). The possibility of infection occurring by drinking milk necessitates the pasteurization or boiling of milk.

**Conclusion:** Bovine brucellosis was prevalent and widely distributed at Chittagong. Commercial farms were at higher risk of brucellosis. The allied risk factors for the occurrence of brucellosis in the study area were overlooked. This research will address the problem of brucellosis to all the farms in Bangladesh, and in Chittagong, in particular. Further studies are needed to identify species and biovars, understand the dynamics of transmission cycles and to identify alternative management practices to replace those that are risk factors for animal and human infections.

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