

## Seroprevalence of Brucellosis and Its Associated Risk Factors in Bovine at Greater Mymensingh District of Bangladesh

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### ABSTRACT

Brucellosis is a zoonotic bacterial disease of humans and animals caused by Gram negative bacteria of the genus *Brucella*. The study was conducted to determine the sero-prevalence of brucellosis and its associated risk factors in cattle and buffalo in greater Mymensingh district, Bangladesh. Blood samples were collected from cattle (n=150) and buffalo (n=60). Sera were tested for *Brucella* specific antibody by the rose Bengal plate test (RBPT). Overall prevalence of brucellosis was 15.33% in cattle and 13.33% in buffalo. Higher prevalence was recorded in cattle and buffalo of over 4 years age (18% and 15.68%, respectively). Female animals showed higher brucellosis prevalence (cattle 19.54%, buffalo 13.46%) than male (cattle 8.21%, buffalo 12.5%). Pregnant cows showed higher prevalence of brucellosis (13.33%) as compare to non pregnant cows (10%). The prevalence of brucellosis was 10% in aborted cows, 4% in the case of retained placenta and 2.85% in repeat breeder cows. The study suggests that brucellosis is prevalent in the cattle and buffalo and its prevalence is affected by the animal's age, sex, pregnancy status and reproductive disorders.

**Keywords:** Seroprevalence, Brucellosis, *Brucella*, Cattle, Buffalo, Risk factor

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### Introduction

Brucellosis is a zoonotic disease caused by non-motile, coccobacilli, Gram-negative bacteria of the genus *Brucella* which show strong host preference. The species of *Brucella* which infect livestock and their primary hosts are: *B. abortus* (cattle), *B. melitensis* (goat), *B. ovis* (sheep), *B. suis* (pig) (Islam *et al.*, 2013). Brucellosis in domestic water buffalo (*Bubalus bubalis*) is mainly caused by *B. abortus* (Brahmabhatt *et al.*, 2009).

Brucellosis is essentially a disease of sexually mature animals (Radosi $\acute{t}$ s *et al.*, 2007). In male animals it causes infertility (Boschiroli *et al.*, 2001; Gwida *et al.*, 2010). In female animals, the most prominent clinical sign of brucellosis is abortion. *Brucella* localize in the udder of the infected cattle and excretes via milk (England *et al.*, 2004). Brucellosis is transmitted by direct or indirect contact with infected animals "often via ingestion and also via venereal routes" (Quinn *et al.*, 1994). Other clinical signs of brucellosis in animals are repeat breeding, retained placenta and metritis (Shareef, 2006).

The epidemiology of brucellosis is complex and it is influenced by several factors (Nicoletti, 1980). Brucellosis is an occupational zoonosis that mainly infect livestock farmers and their families, abattoir workers, farm labors, slaughter-house workers, butchers and veterinarians (Yagupsky and Baron, 2005; Tabak *et al.*, 2008; Behzadi and Mogheiseh, 2011).

The human brucellosis which is also called undulant or Malta fever, is a serious public health problem and has been reported in

many parts of the world such as; Asia, India, Middle Eastern, Southern European, and Latin American countries (Montanaro *et al.*, 1992; Collier *et al.*, 1998).

Bovine brucellosis has emerged as a serious animal and public health issue in many parts of the world (Corbel, 1997). It causes economic losses due to abortion or stillbirth, irregular breeding and loss of milk production especially in countries where rural income relies largely on livestock breeding and dairy products (Maadi *et al.*, 2011). It is one of the most devastating trans-boundary animal diseases and also a major barrier for trade (Gul and Khan, 2007).

Brucellosis is endemic in Bangladesh (Amin *et al.*, 2005; Das *et al.*, 2008) where approximately 80% people live in villages, and the rural income is largely dependent on livestock. Peoples remain in close contact with domestic animal population owing to their occupation and face a constant risk of acquiring brucellosis.

The present study was undertaken to determine the seroprevalence of brucellosis in cattle and buffalo in Mymensingh, Tangail and Sherpur districts and its associated risk factors. Isolation of the *Brucella* spp. from the seropositive and aborted animals was also attempted.

### Materials and Methods

The study was conducted for a period of 10 months (July 2011 to May 2012) at the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh.

#### Sample collection

Venous blood samples were aseptically collected from cattle (n=150) and buffalo (n=60) at breeding farm, Tangail, BAU dairy farm, Char Nillukia, Mymensingh sadar and Char Basur Algi,

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Nokla, Sherpur. Fetal stomach contents, fetal spleens, lymph nodes and lungs were aseptically collected from three aborted cows.

#### Serological test

Rose Bengal plate test (RBPT) was used to detect *B. abortus* specific antibody in the serum samples. The antigen (*B. abortus* strain 119-3) was obtained from the laboratory of Veterinary Public Health, College of Veterinary Medicine, Chonbuk National University, Republic of Korea. The test was performed according to the standard procedures of OIE (2008). The test and control sera were homogenized using a vortex and 10 µl of each serum was placed on a glass plate marked with circles of approximately 2 cm in diameter. After gently shaking the antigen vial 10 µl of antigen was placed beside the serum drop. The antigen and serum were mixed on the plate for exactly 4 min. Definite clumping/ agglutination was considered as a positive reaction, while no clumping/agglutination was the indication of negative reaction.

#### Bacteriological study

Blood samples of seropositive cattle (n=23) and buffalo (n=8) and specimens of fetal stomach contents, spleens, lymph nodes and lungs collected from three aborted animals were cultured on blood agar and brucella agar media for isolation of *Brucella* spp.

The tissue samples were processed according to the procedures described by Alton *et al.* (1975). Blood samples were processed by the lysis concentration method (Kolman *et al.*, 1991) with some modifications. Briefly, 100 µl blood sample was mixed with 900 µl distilled water in an Eppendorf tube. Hemolysed blood samples were centrifuged at 1500 rpm for 30 minutes at 4°C temperature. Supernatant was inoculated duplicate in blood agar and brucella agar media plates and incubated at 37°C for 5 days under 5% CO<sub>2</sub> atmosphere.

Fetal lymph nodes, lungs, spleens were also processed similarly after grinding in a mortar with pestle.

#### Statistical analysis

Data were analyzed for statistical significance by Chi-square test (SPSS 11.5, UK). A *p* value of ≤ 0.05 was considered as statistically significant.

## Results

The overall prevalence of brucellosis was 15.33% in cattle and 13.33% in buffalo. The highest prevalence was recorded in the cattle breeding farm, Tangail (22%) followed by Char Basur Algi, Nokla, Sherpur (16.66), BAU dairy farm (5%) and Char Nillukia (5%) (Table 1).

Prevalence of brucellosis was found to increase with the increase of animal's age (Table 2). The highest prevalence was recorded in cattle and buffalo over 4 years of age (18% and 15.68%, respectively).

A higher prevalence of brucellosis was recorded in female as compared to male. In female cattle, the prevalence of brucellosis was 19.54% against 8.21% in male (*p* < 0.213). Similarly in female buffalo the prevalence was 13.46% while in males it was 12.5% (*p* < 0.314).

Out of 150 cattle, 60 were pregnant and 90 were non-pregnant. The prevalence of brucellosis was 13.33% in pregnant cattle and 10% in non-pregnant cattle. Among the cattle with the history of reproductive disorder, the animals that experienced abortion showed the highest prevalence of brucellosis (10%) followed by those with retained placenta (4%) and repeat breeder (2.85%).

*Brucella* organisms were not isolated from blood samples of the animals as well as from the aborted tissue samples.

**Table 1. Prevalence of brucellosis in cattle and buffalo**

Animal species	Study areas	No. of sera tested	No. of positive reactors (%)	p value
Cattle	Breeding farm, Tangail	50	11 (22)	0.247
	BAU Dairy Farm	20	1 (5)	
	Char Nillukia, Mymensingh	20	1 (5)	
	Char Basur Algi, Nokla, Sherpur	60	10 (16.66)	
Buffalo	Breeding Farm, Tangail	60	8 (13.33)	0.612

BAU = Bangladesh Agricultural University

**Table 2. Prevalence of brucellosis in cattle and buffalo of different age groups**

Animal species	Age of animals	No. of sera tested	No. of positive reactors (%)
Cattle	3 month- 1 year	35	4 (11.42)
	1 – 2 years	24	2 (14.28)
	2 -4 years	41	7 (17.07)
	Above 4 year	50	9 (18)
Buffalo	6 month – 2 years	4	0 (0)
	2 – 4 years	5	0 (0)
	Above 4 years	51	8 (15.68)

## Discussion

This study recorded 15.33% prevalence of brucellosis in cattle. Prevalence of brucellosis recorded in this study disagreed with the findings of Amin *et al.* (2004), Rahman *et al.* (2006) and Rahman *et al.* (2012) who reported 2%, 3.8% and 2.66% prevalence of brucellosis in cattle in Mymensingh and Sherpur districts of Bangladesh. This variation of prevalence might be due to the difference of animal's age, sex, breed, pregnancy status and the study area, animal management practice, herd size, reproductive diseases and serological tests applied (Gul and Khan, 2007; Kebede *et al.*, 2008). The present study recorded 22% prevalence of brucellosis in cattle at the breeding farm, Tangail. Rahman *et al.* (1978) have reported 11.44% prevalence of brucellosis in Savar dairy farm, 16.16% in Tangail, and 30.7% in Pabna milk shed areas of Bangladesh.

In the current study, prevalence of brucellosis in cattle was 17.07% in 2 to 4 years age group and 18% over 4 years age group. The difference of prevalence of brucellosis between these groups was statistically significant (*p* < 0.05). In contrast to the findings of the present study Rahman *et al.* (2011) reported 1.45% prevalence of brucellosis in cows aged 2.5 to 4 years and 3.54% in cows over 4 years of age. Amin *et al.* (2005) reported 2.3% and 4% prevalence in the < 4 and > 4 years age group, respectively. Although susceptibility to brucellosis increases with age, it seems to be more commonly associated with sexual maturity (Radostits *et al.*, 2007).

Brucellosis is known to cause abortion, retention of placenta, repeat breeding, infertility and prolonged inter-calving period in animals (Radostits *et al.*, 2007). This study recorded 10% prevalence of brucellosis in cattle with history of abortion, 4% prevalence in retained placenta and 2.85% in repeat breeder cattle. Bachh *et al.* (1988) reported 89% prevalence of brucellosis in cattle with a history of abortion. Rahman *et al.* (2006) recorded 15% prevalence of brucellosis in aborted cows and 1.45% in repeat breeder cows and 13.04% in cows with the history of retained placenta. Ibrahim and Habiballa (1975) reported 14.2% prevalence of brucellosis in cows with history of abortion.

Rahman *et al.* (2006) reported 13.04% prevalence of brucellosis in cows with a history of retained placenta.

In this study, *Brucella* spp. was not isolated from any of the aborted fetal tissues. Sera obtained from three aborted cattle did not react in RBPT indicating that abortion might not have been caused by *Brucella* spp. The present study did not isolate *Brucella* spp. from any of the *Brucella* seropositive blood samples. Ganado and Bannister (1960) noticed suboptimal recovery rate of *Brucella* from blood samples. Seropositive animals sometimes yield negative culture results (Alton *et al.*, 1988).

This study recorded 13.33% seroprevalence of brucellosis in buffalo. Rahman *et al.* (2012) reported 8.33% prevalence of brucellosis in buffalo in Mymensingh district. In the present study, buffalo over 4 years of age had higher prevalence (15.68%) than other age groups. Similar observations were also recorded by Vikrant *et al.* (2006) and Puspha and Kumari (2005). Control of brucellosis in animal help reduce the prevalence of brucellosis in humans (WHO, 1981). Therefore, regular surveillance of brucellosis in domesticated livestock is essential in order to undertake prevention and control measures. RBPT is easy to perform and very helpful in screening and monitoring brucellosis in cattle (OIE, 2008).

## Conclusions

The findings of this study, suggested that brucellosis is endemic in cattle and buffalo in the study areas. However, more surveys are required across the country in order to formulate a policy for prevention and control of brucellosis in livestock and human population.

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