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ORIGINAL ARTICLE

Effects of seasonal, environmental, and demographic factors on brucellosis in buffaloes in Bhola District, Bangladesh

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Abstract

Background: Brucellosis is a contagious zoonotic disease that poses a major threat to livestock health and productivity, especially in buffaloes. In Bangladesh, information on its prevalence in coastal regions such as Bhola—where traditional Bathan-based buffalo farming is practiced—is limited. Seasonal variation, water source, grazing patterns, and demographic factors such as age, sex, and body condition score (BCS) may influence disease occurrence. Understanding these determinants is essential for designing effective control strategies for the region.

Methods: The study was conducted in Bhola District, Bangladesh, from January 1 to December 30, 2024. A total of 150 buffaloes were screened for brucellosis using the Rose Bengal Plate Test (RBPT) and indirect ELISA (i-ELISA). Information on age, sex, BCS, season, water source, grazing area, and Bathan hygiene was collected through structured interviews and direct observation.

Results: The overall seroprevalence of brucellosis was 8% by RBPT and 6.67% by i-ELISA. Although female buffaloes aged 2–4 years showed a higher prevalence (7.37%) than males (3.57%), and animals with a low BCS (1–2) had the highest infection rate (11.90%), these differences were not statistically significant. Prevalence was highest during the rainy season (12%), and buffaloes consuming river or pond water had higher seropositivity (7.92%) compared with those using tube-well water (4.08%). Buffaloes grazing in altered grazing areas (7.14%) and those kept in poorly managed Bathans (7.96%) also showed higher prevalence than their counterparts; however, none of these associations reached statistical significance.

Conclusion: Although variations in seroprevalence were observed across sex, age, body condition, season, water source, grazing practices, and Bathan hygiene, none of these factors showed statistically significant associations with brucellosis in buffaloes in Bhola. These descriptive findings nonetheless suggest potential risk patterns and underscore the importance of continuous surveillance, good husbandry practices, and farmer awareness to minimize the risk of brucellosis and its economic impact in the region.

Keywords: Brucellosis. seroprevalence, environmental and managemental factors, Bhola

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Introduction

Livestock is an essential component of the farming system in Bangladesh, providing meat, milk, draft power, transportation, and manure-based fertilizer, while also generating employment. The livestock subsector offers full-time employment for 20% of the population and part-time employment for another 50% (Rahman *et al.*, 2014b). However, its total economic contribution is often underestimated, as values added through draft power, threshing, oilseed crushing, local transport, cooking fuel, and manure for crop production are rarely included in national assessments. Human livelihoods in Bangladesh remain closely linked to livestock across various production systems (Bekele Megersa *et al.*, 2011). Despite its importance, the sector faces multiple threats, including brucellosis—a highly infectious and contagious zoonotic disease affecting a wide range of domestic animals and humans (Rahman *et al.*, 2011).

Previous studies reported a seroprevalence of 2.87% in buffaloes from selected regions of Bangladesh (Rahman *et al.*, 2011), while a global meta-analysis estimated the worldwide prevalence in buffaloes at 9.7% (Shi *et al.*, 2021). Higher regional prevalence has been documented, such as 14.2% in India (PA *et al.*, 2023). Climatic and seasonal factors have been identified as major determinants of human brucellosis in China (Liu *et al.*, 2020), and *Brucella* organisms can persist for extended periods in cold and humid environments, facilitating transmission across diverse geographic settings (Aune *et al.*, 2012).

Common serological tests for detecting *Brucella* antibodies include RBT, SAT, TAT, the mercaptoethanol test, and ELISA. Among these, ELISA offers superior sensitivity and is useful for determining infection status regardless of vaccination history (Sousa *et al.*, 2017). Despite the significance of buffalo brucellosis, limited epidemiological studies have been conducted in Bangladesh. Therefore, a study investigating the effects of seasonal, environmental, and demographic variables on the seroprevalence of brucellosis in buffaloes in the study area is warranted.

Materials and methods

Study area and duration

This study was conducted in the central coastal belt of Bhola District, Bangladesh, to evaluate the effects of seasonal, environmental, and demographic variables on the seroprevalence of brucellosis in buffaloes. The investigation covered a full calendar year, from January 2024 to December 2024.

Selection of the farm and buffalo

A total of 150 buffaloes were purposively selected from four Bathans located in Bhola Sadar Upazila, Bhola (Figure 1). Purposive sampling was adopted to account for the heterogeneity in buffalo management practices across the study areas and to ensure representation of different Bathan-based production systems.

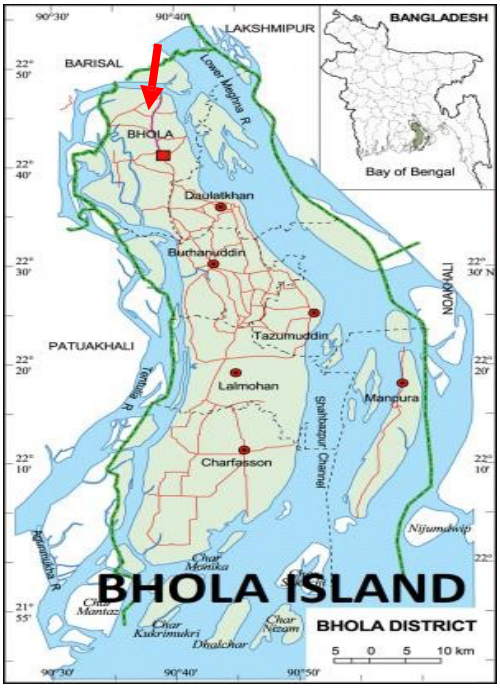

Figure 1. Red arrow marking the location of the study area (https://tiermaker.com/categories/geo-maps)

The required sample size was calculated using the standard formula for estimating prevalence in cross-sectional studies:

$$n = \frac{Z^2 \times P \times (1-p)}{d^2}$$

where Z represents the Z-score for a 95% confidence level (1.96), P denotes the expected prevalence (10% or 0.10), and d indicates the desired margin of error (5% or 0.05). Using these parameters, the estimated sample size was approximately 150 buffaloes.

Diagnosis of the disease

Brucellosis in buffaloes was diagnosed through clinical examination, the Rose Bengal Plate Test (RBPT), and the Enzyme-Linked Immunosorbent Assay (ELISA).

Clinical Examination

Prior to clinical examination, information on the owner's observations and concerns regarding the animals was recorded. A detailed disease history was obtained by interviewing the owners, farmers, or attendants. The animals were then visually examined for physical condition, as well as signs of abortion, stillbirth, weak calves, and other systemic abnormalities.

Serological test

i) Rose Bengal Plate Test

The RBPT was performed following the manufacturer's instructions (Lillidale Diagnostics, UK). Sera and antigens were equilibrated to room temperature. Equal volumes (30 µL) of standardized *B. abortus* antigen strain 99 and test serum were mixed on a glass plate and agitated for 2–5 minutes. Agglutination observed within 1 minute was considered positive, while the absence of agglutination after 2–5 minutes was considered negative.

ii) Enzyme-Linked Immunosorbent Assay (ELISA)

The ID Screen Brucellosis Serum Indirect Multispecies kit (Innovative Diagnostics, Grabels, France) was used to detect antibodies against *B. abortus* (bovine), *B. melitensis* (ovine and caprine), and *B. suis* (porcine), following the manufacturer's protocol.

Statistical analysis

Data were entered into Microsoft Excel 2019, checked for errors, and corrected as necessary. Seroprevalence was calculated by dividing the number of positive samples by the total number of animals tested. Univariable associations between seropositivity and potential risk factors were assessed using the chi-square test. Binary and multivariable logistic regression analyses were conducted to identify significant risk factors for brucellosis. A p-value ≤ 0.05 was considered statistically significant, with a 95% confidence level and 5% precision.

Results and discussion

Overall prevalence of brucellosis in buffaloes

Brucellosis is the second most frequently reported zoonotic disease according to the World Organization for Animal Health (OIE) and is considered one of the most devastating transboundary animal diseases (Lokamar *et al.*, 2022). In the present study, the overall prevalence of brucellosis in buffaloes was 8% and 6.67% using the RBPT and i-ELISA tests, respectively (Table 1). Previous studies in Bangladesh reported lower prevalence rates, with 2.96% and 1.48% using RBPT and i-ELISA, respectively (Rahman *et al.*, 2012). Conversely, other studies reported higher prevalence, such as 13.46% in selected regions of Bangladesh (Islam *et al.*, 2013). Regional variations in prevalence have also been observed in Dhaka, Chittagong, and Sylhet (Deb Nath *et al.*, 2023; Islam *et al.*, 2021; Rahman *et al.*, 2019). These differences may be attributed to variations in geographical location, management practices, sampling strategies, diagnostic methods, host resistance, and natural immunity levels

Seroprevalence of brucellosis according to demographic factors

In the present study, sex-wise analysis showed a higher prevalence of brucellosis in female buffaloes (7.37%) compared to males (3.57%). Although females exhibited higher positivity, the association was not statistically significant (Tables 2 and 3). This higher prevalence in females may be due to the aggressive replication of Brucella bacteria within placental trophoblasts, leading to reproductive tract disorders and late-term abortions (Roop *et al.*, 2009). Interestingly, Rahman *et al.* (2012) reported the opposite trend, with a higher prevalence in males (7.14%) than in females

(1.87%). Conversely, another study supported the present findings, showing higher infection rates in females (6.82%) compared to males (3.85%) (Rahman *et al.*, 2014a). Age-wise prevalence in the present study indicated that the highest brucellosis rates were observed in buffaloes aged >2–4 years (9.09%), followed by >4 years (6.02%), while animals aged 1–2 years showed no positive cases. Although older animals (>2 years) had higher positivity than younger ones, these associations were not statistically significant (Tables 2 and 3). This is consistent with Rahman *et al.* (2012), who also reported the highest prevalence in buffaloes aged >2–4 years (4.17%). In contrast, Islam *et al.* (2012) found the highest prevalence in animals over 4 years of age (15.68%). Similarly, studies from Pakistan and India reported higher brucellosis prevalence in buffaloes older than 2 years (12.50% in Pakistan; 8.24% in India) compared to younger groups (2.98% and 8.27%, respectively) (Muthiah *et al.*, 2024). The increased prevalence in older animals may be attributed to declining immunity and greater cumulative exposure to the pathogen over time. Regarding body condition score (BCS), the highest prevalence was observed in buffaloes with poor body condition (BCS 1–2, 11.90%), followed by BCS >2–3 (5%) and BCS >3–4 (3.57%). Although animals in better condition had lower odds of infection, these differences were not statistically significant (Tables 2 and 3). Similar trends have been reported in Africa, where buffaloes with poor health were significantly more likely to be infected (Gorsich *et al.*, 2015). Supporting this, studies in cattle have shown higher brucellosis prevalence among animals with reduced body condition (Abera *et al.*, 2019; Etefa *et al.*, 2022). These findings suggest a potential link between brucellosis and lower body condition in buffaloes, though further research is needed to establish the prevalence across different BCS categories in domestic water buffaloes (Bubalus bubalis) in various regions.

Table 1. Overall prevalence of brucellosis in buffaloes

Species	**Number of sera tested**	**Seropositive no. (%)**		**χ^2**	**p-value**
		RBPT	I-ELISA	0.196207	0.65
Buffalo	150	12 (8.00%)	10 (6.67%)		

Table 2. Prevalence of brucellosis in buffaloes according to different demographic variables in Bhola district, Bangladesh.

Risk factors	**Category**	**N**	**Positive No. (%)**	**χ^2**	**P value**
Sex	Male	28	1 (3.57%)	0.669643	0.41
	Female	122	9 (7.37%)		
Age	1-2 years	12	0(0.00%)	1.431701	0.48
	>2-4 years	55	5(9.09%)		
	>4 years	83	5(6.02%)		
Body Condition Score (BCS)	1-2	42	5(11.90%)	0.988526	0.61
	>2-3	80	4(5%)		
	>3-4	28	1(3.57%)		

Table 3. Binary logistic regression of demographic factors associated with brucellosis prevalence in buffaloes

Risk Factor	Category	N	Positive (%)	Odds Ratio (OR)	95% CI	P-value
Sex	Male	28	1 (3.57%)	Ref	-	-
	Female	122	9 (7.37%)	2.12	0.25–17.8	0.41
Age	1-2 years	12	0 (0.00%)	Ref	-	-
	>2-4 years	55	5 (9.09%)	1.67	0.08–34.7	0.48
	>4 years	83	5 (6.02%)	1.00	0.05–21.3	0.48
BCS	1-2	42	5 (11.9%)	Ref	-	-
	>2-3	80	4 (5.0%)	0.39	0.08–1.93	0.61
	>3-4	28	1 (3.57%)	0.28	0.03–2.92	0.61

Bovine brucellosis in buffaloes according to seasonal and environmental effects

The present study reported that the prevalence of brucellosis was highest during the rainy season (12%), followed by summer (5.77%) and winter (3.85%). Although the seasonal differences were not statistically significant, a clear trend of higher prevalence in the rainy season was observed (Tables 4 and 5). There is no previous data on the seasonal prevalence of brucellosis in Bangladesh; however, these findings align with reports from Pakistan, where regions with higher rainfall and humidity showed increased Brucella infection in buffaloes (Abubakar *et al.*, 2010). Similarly, studies in China indicate that climatic factors, including temperature, humidity, and wind speed, significantly influence brucellosis transmission, with higher temperatures and humidity enhancing bacterial survival and spread (Chen *et al.*, 2023). Mathematical modeling in Tanzania also demonstrated that weather variations affect disease transmission among humans, wildlife, and livestock, highlighting the importance of timely interventions in response to seasonal variation (Nyerere *et al.*, 2020). Regarding water sources, buffaloes that drank pond or river water (7.92%) were more frequently infected than those using tube well water (4.08%). Although the difference was not statistically significant (Tables 4 and 5), this finding suggests that clean water may reduce the risk of brucellosis. Contamination of water with reproductive discharges from aborted fetuses or uterine secretions can facilitate pathogen transmission among animals (Rahman *et al.*, 2012). Proper management and monitoring of water sources are therefore essential. Studies have reported that 85% of buffalo keepers relied solely on water to clean cattle sheds without disinfectants,

which may be insufficient to remove pathogens (Kant *et al.*, 2018). Climate change and prolonged droughts further reduce water availability, forcing farmers to use suboptimal sources that may compromise livestock health and increase disease risk (Gould *et al.*, 2015; Robi *et al.*, 2023). Buffaloes managed under altered grazing systems showed a higher prevalence of brucellosis (7.14%) than those under regular grazing (5.26%), although the difference was not significant (Tables 4 and 5). Traditional communal grazing and altered livestock movements can increase brucellosis risk in Bangladesh (Rahman *et al.*, 2024). Climate change can degrade grazing lands, forcing animals into new areas where exposure to pathogens is higher. Altered grazing patterns may also increase wildlife–livestock interactions, intensifying the risk of zoonotic disease spillover (Escarcha *et al.*, 2018; Ekwem *et al.*, 2021; Karmacharya *et al.*, 2024). Peak disease incidence often coincides with the end of grazing periods, indicating that movement timing plays a key role in disease transmission (Knific *et al.*, 2020). Hygienic management of animal sheds also influenced brucellosis prevalence. Buffaloes housed in more hygienic sheds showed lower infection rates (2.70%) compared to those in poorly maintained conditions (7.96%), although the association was not statistically significant (Tables 4–6). Maintaining clean living conditions, including proper disposal of aborted fetuses, placental tissues, and uterine discharges, is crucial for reducing environmental contamination and controlling brucellosis (Tabar and Jafari, 2014). Restricting animal movement from infected areas further reduces transmission risk (H Abd-El Halim *et al.*, 2017). Globally, the implementation of comprehensive control measures has effectively reduced brucellosis prevalence in buffaloes, with seroprevalence declining from 20.8% before 2010 to 4.2% between 2010 and 2020, demonstrating the success of improved hygiene, management, and control strategies.

Table 4. Prevalence of brucellosis in buffaloes in relation to seasonal and management factors in Bhola District, Bangladesh

Variable	Category	N	Positive No. (%)	χ^2	P value
Season	Summer	52	3 (5.77%)	0.721	0.697
	Rainy	72	6 (12%)		
	Winter	26	1 (3.85%)		
Sources of drinking water	River/pond water	101	8 (7.92%)	0.781	0.376
	Tubewell water	49	2 (4.08%)		
Altered grazing system	Yes	112	8 (7.14%)	0.161	0.688
	No	38	2 (5.26%)		
Hygienic management	Yes	40	1 (2.70%)	1.240	0.265
	No	110	9 (7.96%)		

Table 5. Binary logistic regression analysis of seasonal and managemental risk factors for brucellosis seroprevalence in buffaloes

Variable	Category Compared	Odds Ratio	95% CI	P value
Season	Rainy vs. Summer	2.62	0.59-11.6	0.27
	Winter vs. Summer	0.65	0.06-7.34	0.70
Water Source	Tubewell vs. River/Pond	0.49	0.10-2.50	0.39
Altered grazing	No vs. Yes	0.72	0.14-3.66	0.69
Hygienic management	No vs. Yes	3.12	0.37-26.3	0.24

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Conflict of interest

The authors declare that they have no conflict of interest.

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