Original Article

Outbreak Investigation of Hemorrhagic Septicemia in Water Buffalo on Meghna River Island, Bhola, Bangladesh

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How to Cite This Article Khalil, I., Sayed, A., Jawad, N., Mamun, T. I., Mahmud, Sh., Rahman, Kh., & Hayat, S., et al. (2026). Outbreak Investigation of Hemorrhagic Septicemia in Water Buffalo on Meghna River Island, Bhola, Bangladesh. *Iranian Journal of Veterinary Medicine, 20*(1), 67-76. http://dx.doi.org/10.32598/ijvm.20.1.1005792

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ABSTRACT

Background: Hemorrhagic septicemia (HS), caused by *Pasteurella multocida* serotype B:2, is a fatal bacterial disease of water buffaloes, particularly threatening in ecologically vulnerable coastal regions such as Bhola, Bangladesh.

Objectives: This study reports an HS outbreak on Meghna River Island from March 17 to April 6, 2023, focusing on epidemiological patterns, clinical manifestations, and molecular diagnostics.

Methods: This is a cross-sectional observational study that was conducted on a herd of buffaloes on a farm in Vabanipur Char (Island), Bangladesh, that exhibited sudden illness characterized by high fever, respiratory distress, and lethargy. Among 200 buffaloes, 51 developed clinical signs and 19 died. During the field visits, blood samples were collected from four clinically affected live buffaloes for bacteriological analysis. Additionally, three blood and three lung tissue samples were obtained from the deceased animals for comprehensive diagnostic evaluation.

Results: The outbreak exhibited a morbidity rate of 25.5%, a mortality rate of 9.5%, and a case fatality rate of 37.3%. Males showed 100% morbidity, whereas females had the highest case fatality rate (46.7%). Predominant clinical signs included high fever, respiratory distress, bloat, and swallowing difficulties—strongly associated with fatal outcomes. Postmortem findings revealed marked subcutaneous edema and visceral organ congestion. Molecular detection using quantitative real-time PCR targeting the Kmt1 gene confirmed *P. multocida* in all tested samples.

Article info:
Received: 28 Apr 2025
Accepted: 16 Jul 2025
Publish: 01 Jan 2026

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Conclusion: The findings underscore the impact of climatic stressors on HS emergence and highlight the urgent need for robust vaccination programs, timely diagnosis, and effective disease surveillance systems in coastal livestock populations. Future studies should focus on genetic characterization and long-term control strategies in climate-sensitive zones.

Keywords: Hemorrhagic septicemia, Meghna River Island, *Pasteurella multocida*, Outbreak investigation, Water Buffalo

Introduction

Hemorrhagic septicemia (HS) is a highly contagious and fatal bacterial disease caused by *Pasteurella multocida*, primarily affecting water buffaloes and cattle (Almoheer et al., 2022). While both species are susceptible, buffaloes experience a more severe form of the disease, with untreated cases frequently resulting in death (Cuevas et al., 2020). The causative agent, *P. multocida*, is a gram-negative, non-motile bacterium that normally resides in the respiratory tracts of healthy animals. However, under stressful conditions or immunosuppression, it can be transformed into a pathogenic state, leading to systemic infections (Ginting et al., 2022). The disease progresses rapidly, manifesting with clinical signs, such as fever, depression, submandibular edema, and dyspnea. In buffaloes, death can occur within 24 to 48 hours of symptom onset, leaving minimal time for effective treatment (Almoheer et al., 2022; Ara et al., 2016).

Early and accurate diagnosis of HS is crucial for controlling outbreaks. Assessing clinical signs and postmortem examinations are the primary diagnostic approaches. Isolation of *P. multocida* from blood or tissue samples, biochemical tests, and polymerase chain reaction (PCR) assays provide definitive confirmation (Moustafa et al., 2017). Additionally, in field settings, impression smears stained with Leishman's stain can serve as a rapid preliminary diagnostic tool (Khan et al., 2011). Despite the availability of these techniques, timely diagnosis remains a challenge, particularly in remote areas where access to veterinary services is limited. Furthermore, although vaccination is the most effective preventive measure against HS, logistical constraints related to distribution, storage, and administration significantly hinder its widespread use in rural regions (Shivachandra et al., 2011).

In Bangladesh, water buffaloes play a crucial role in the livelihoods of rural communities, particularly on riverine islands, like Bhola, situated in the Meghna River basin. The island's unique environmental conditions—including seasonal flooding, tidal surges, and high humidity—create an ecosystem conducive to disease outbreaks. These factors, coupled with overcrowding of livestock during flooding and restricted access to veterinary care, exacerbate the spread of HS. The disease progresses swiftly, often resulting in near 100% mortality if left untreated (Shome et al., 2024), and poses a severe threat to the local buffalo population. Moreover, buffaloes are more vulnerable to HS than cattle due to the accelerated disease course, making early detection and intervention critical.

Despite the well-documented impact of HS on livestock, its epidemiology in Bangladesh—particularly on Bhola Island—remains poorly understood. To bridge this knowledge gap, the present study investigated an HS outbreak in a water buffalo herd on Meghna River Island. Specifically, it confirmed the presence of *P. multocida* through both classical and molecular techniques, and assessed the demographic characteristics of affected animals to understand the overall epidemiology of this disease in this area. By providing novel epidemiological insights, this study aimed to enhance prevention and control strategies, ultimately safeguarding buffalo populations and supporting the livelihoods of rural communities.

Materials and Methods

Outbreak notification and field investigation

The outbreak began on March 17, 2023, when several buffaloes on a farm in Vabanipur Char (Island) exhibited sudden illness characterized by high fever, respiratory distress, and lethargy (Figure 1). In response, the farmer initially administered a combination of commonly used veterinary drugs to the animals, including antibiotics, such as oxytetracycline (10 mg/kg body weight [BW]), enrofloxacin (2.5–5 mg/kg BW), sulphadimidine (100–150 mg/kg BW), and procaine penicillin (20,000–40,000 IU/kg BW), antihistamines, such as chlorpheniramine maleate (0.5–1 mg/kg BW), antipyretics, such as paracetamol (10–15 mg/kg BW) and flunixin meglumine (1.1–2.2 mg/kg BW), corticosteroids, like dexamethasone (0.1 mg/kg BW), and intravenous isotonic saline.

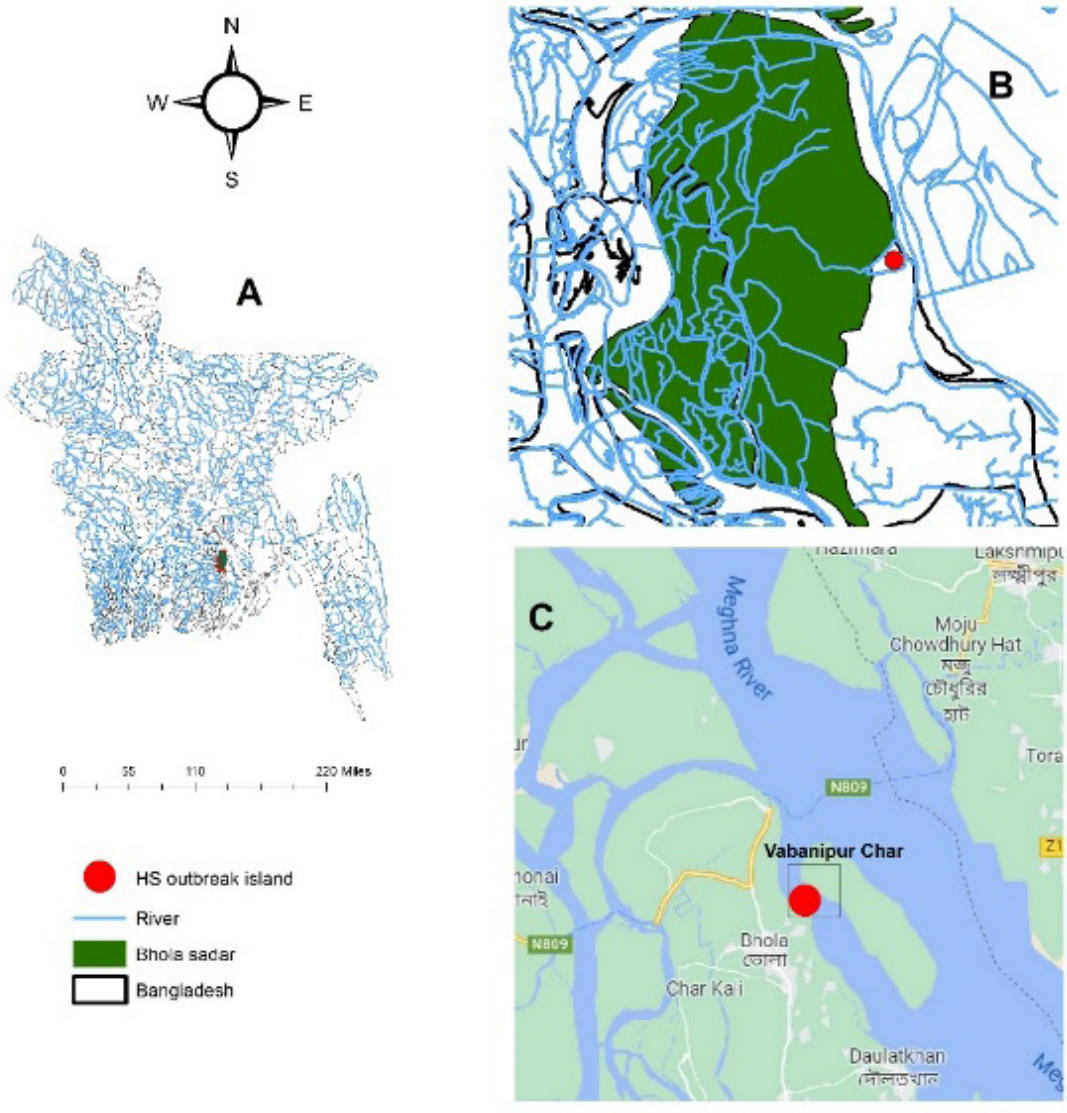

Figure 1. Study location of the HS outbreak investigation in water buffaloes on Vabanipur River Island in Bhola

Despite this comprehensive therapeutic approach, the animals showed no significant improvement, and new cases continued to emerge. As the situation deteriorated, the farmer notified the Upazila (sub-district) Livestock Office (ULO) on March 24, 2023. Upon observing continued morbidity and poor clinical response to treatment, the ULO reported the case to the Field Disease Investigation Laboratory (FDIL), Barishal, a regional animal health laboratory under the Government of Bangladesh, on March 27, 2023.

In response, an outbreak investigation team was immediately formed, comprising an epidemiologist, a laboratory technician, and a local animal health official cum veterinary doctor, under the supervision of the principal scientific officer of FDIL, Barishal. The team visited the affected site on March 28, 2023, to conduct a comprehensive field investigation and assess the extent of the outbreak. All relevant data and clinical specimens were collected during the field visit and post-mortem examination for further laboratory analysis.

Vabanipur Char is a riverine island located within the Meghna River system (22.732266°N, 90.656057°E), covering approximately 260 acres, of which about 50 acres were allocated for buffalo farming by a single herd owner. Environmentally, the area is frequently exposed to climate-related stressors, such as salinity intrusion, elevated ambient temperatures, and erratic rainfall patterns—factors known to influence the emergence and transmission of infectious diseases. Notably, in the days preceding the outbreak, the region experienced a period

of intense heat followed by abrupt heavy rainfall, which may have imposed substantial physiological stress on the animals, predisposing them to disease.

Although a formal record-keeping system was not maintained, the farmer had given unique names to each buffalo. Together with his caretaking team, he was able to recall the clinical history of each animal, thereby enabling accurate tracking of disease onset, progression, and outcomes at the individual level.

Study design and period

This cross-sectional observational study was conducted from March 19 to April 4, 2023. This study period encompassed both the peak of the outbreak and the early recovery phase, allowing for a comprehensive assessment of disease transmission dynamics, clinical outcomes, and the effectiveness of immediate field interventions.

Clinical observations

The affected herd consisted of 200 water buffaloes, among which 51 animals developed clinical signs consistent with HS during the outbreak period. Nineteen buffalo succumbed to the disease. Notably, none of the animals had been vaccinated against HS in the preceding two years, leaving the population highly susceptible to infection. Clinical signs observed in affected animals included fluctuating high fever (≥39.5 ˚C), abdominal bloat, submandibular edema, respiratory distress, nasal discharge, regurgitation, and sudden death in acute cases. A qualified veterinarian conducted detailed clinical examinations, while postmortem inspections were performed on deceased animals to identify gross pathological lesions characteristic of HS.

Case definition

A case of HS was defined as any buffalo exhibiting high fever, respiratory distress, nasal discharge, dysphagia, subcutaneous edema—particularly in the submandibular region—or sudden death. Cases were further categorized as suspected or confirmed. Suspected cases were those showing clinical signs indicative of HS, whereas confirmed cases were defined by positive identification of *P. multocida* through bacteriological culture, postmortem lesions consistent with HS, and molecular confirmation using PCR.

Sample collection and laboratory confirmation

During the field investigation, blood samples were collected from four clinically affected live buffaloes for bacteriological analysis. Additionally, three blood and three lung tissue samples were obtained from the deceased animals for comprehensive diagnostic evaluation. In total, ten specimens were submitted to the Central Disease Investigation Laboratory (CDIL), Dhaka, which operates under the Department of Livestock Services, People's Republic of Bangladesh. All samples were preserved and transported under a strict cold chain protocol to ensure diagnostic integrity for subsequent bacteriological culture and molecular analysis.

Postmortem examination

Postmortem investigations revealed the typical lesions consistent with HS. Marked hemorrhages were observed in the lungs, along with edematous swelling in the throat region and congestion in various visceral organs. The presence of serosanguineous fluids in the thoracic and abdominal cavities was also noted, suggesting a systemic bacterial infection.

Staining procedures

Bacteriological confirmation of *P. multocida* was performed using staining techniques. Gram staining of blood smears revealed the presence of gram-negative, short rod-shaped bacteria. Further confirmation was obtained through bipolar staining using Leishman's and Methylene Blue stains, demonstrating the characteristic "safety-pin" appearance of *P. multocida*. This bipolar morphology is a hallmark of HS-causing *P. multocida* and aids in its differentiation from other bacterial pathogens.

Molecular detection of *P. multocida* using quantitative real-time PCR (qRTPCR)

Microscopic examination of stained blood smears from clinically suspected buffaloes revealed the presence of gram-negative, bipolar-staining short rods suggestive of *P. multocida*. However, due to the limitations of conventional staining methods in differentiating closely related pathogens, molecular confirmation was performed using a TaqMan-based qRT-PCR assay targeting the *Kmt1* gene, a conserved molecular marker specific to *P. multocida* serotype B:2.

Total genomic DNA was extracted from blood and lung tissue samples using the QIAamp DNA Mini Kit (QIAGEN, Germany), following the manufacturer's protocols. DNA quantity and quality were ensured prior to downstream applications.

Table 1. Primer and probe sequences used for taqman-based qPCR targeting the *Kmt1* gene of *P. multocida* serotype B:2

Component	Name	Sequence (5'–3')	Target Position (bp)	Amplicon Size (bp)
Forward primer	PmtKmt_F	CAGAGTTTGGTGTGTTGA	146–163	113
Reverse primer	PmtKmt_R	CAGACTGACAAGGAAATATAAAC	236–258	
TaqMan probe[1]	PmtKmt_pb	FAM–AATC+TGC+TTCCTT+GAC–BHQ1	167–182	

[1]The probe incorporates LNA modifications (indicated by "+") to enhance hybridization stability and specificity. FAM=6-carboxyfluorescein (reporter dye); BHQ1=Black Hole Quencher 1.

qRTPCR was performed using the Bio-Rad CFX Opus Real-Time PCR Detection System. Each 25 µL reaction mixture contained 12.5 µL of GoTaq® qRTPCR Master Mix (Promega, USA), 1 µL of each of forward and reverse primers (10 µM), 5 µL of template DNA, and 5.5 µL of nuclease-free water. The thermal cycling conditions included an initial denaturation step at 95 ˚C for 5 minutes, followed by 40 cycles of denaturation at 94 ˚C for 10 seconds, annealing at 56 ˚C for 20 seconds, and extension at 62 ˚C for 20 seconds.

The primers and probe used in this study were adopted from Settypalli et al. (2016), targeting a 113-bp region of the *Kmt1* gene. The oligonucleotide sequences are provided in Table 1. The probe incorporates locked nucleic acid (LNA) modifications to improve binding stability and detection sensitivity. Positive control reactions utilized a vaccine strain of *P. multocida* serotype B:2 obtained from the Livestock Research Institute (LRI), Bangladesh. No-template controls (NTCs) with nuclease-free water were included in each run to ensure the absence of contamination or non-specific amplification.

Data management and analysis

The collected data were transferred to MS Excel for processing and analysis. Morbidity, mortality, and case fatality rates were calculated for each buffalo category (male, female, calf, heifer, and adult). Age was categorized into three groups: 0–12 months (calves), 13–24 months (heifers), and >24 months (adults), and these were used to analyze age-based differences in mortality. The frequencies of specific clinical symptoms (e.g. fluctuating fever, bloat, and swallowing difficulties) and necropsy findings were recorded. The association between clinical signs and mortality in buffaloes affected by HS was assessed using the Chi-Square statistic, with a significance level set at $P<0.05$. All statistical analyses and visualizations of outbreak patterns and spatial case distribution were carried out using R software (version 4.3.1, 2023) and Microsoft Excel (2016). The geographical maps for the study were generated using ArcGIS version 10.8 (Esri, Redlands, CA, USA).

Results

Epidemiological findings

The study of the HS outbreak on the Meghna River Island in Bhola revealed significant variations in morbidity, mortality, and case fatality rates across different buffalo categories. Among the 200 animals observed, 51 were affected, and 19 succumbed to the disease, resulting in an overall morbidity rate of 25.50%, a mortality rate of 9.50%, and a case fatality rate of 37.25% (Table 2). Male buffaloes exhib-

Table 2. Distribution of HS outbreak on Meghna River Island of Bhola

Parameter	No.			%		
	Animals	Sick Animals	Dead Animals	Morbidity	Mortality	Case Fatality
Male	21	21	5	100	23.8	23.8
Female	179	30	14	16.75	7.82	46.67
Calf (0–12 m)	23	18	5	78.26	21.73	27.78
Heifer (13–24 m)	40	33	14	82.5	35	42.42
Adult (>24 m)	137	0	0	0	0	0
Overall	200	51	19	25.5	9.5	37.25

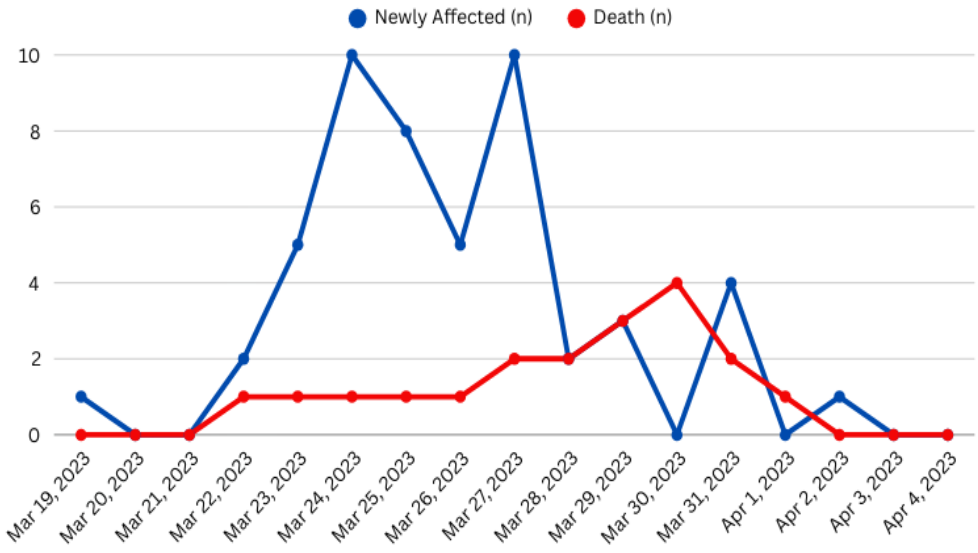

Figure 2. Epidemiological curve of HS in water buffaloes: New cases and deaths (March–April 2023)

ited a 100% morbidity rate and a mortality rate of 23.80%, while females had a lower morbidity rate (16.75%) but a higher case fatality rate (46.67%). Calves and heifers were particularly vulnerable, with morbidity rates of 78.26% and 82.50%, respectively. These findings underscore the outbreak's severity, particularly among females and heifers, which recorded the highest case fatality rates.

Epidemic curve

The index case of the outbreak was reported on March 17, 2023, on Bhola Island. The epidemic curve (Figure 2) illustrated the timeline of the outbreak, spanning from March 17 to April 4, 2023. Initial cases emerged around March 19, followed by a sharp increase between March 21 and March 25, peaking at 11 new cases on March 25. Mortality followed a delayed trend, with deaths increasing significantly around March 25 and peaking at five on March 29. Both new cases and deaths began to decline after March 31, with the outbreak resolving by April 4. The data suggest a rapid disease progression, with new cases surging early in the outbreak and mortality lagging behind.

Clinical and necropsy findings

Buffaloes affected by HS exhibited a sudden onset of clinical signs, including high fever (106 °F), depression, anorexia, watery nasal discharge, cough, and lacrimation. Within 24 hours, respiratory distress developed, characterized by open-mouth breathing and mucoid nasal discharge. By the second day, respiratory distress intensified, leading to recumbency and terminal-stage symptoms, such as extended neck posture and swallowing difficulties. Additional signs included bloat, staggering, and persistent nasal discharge (Table 3).

Despite early intervention with the medications in all the deceased animals for two to three consecutive days, only a transient reduction in fever (96-97 °F) was observed; however, the affected animals ultimately succumbed due to severe dyspnea developed within the next three to four days. Statistical analysis identified fluctuating fever (P=0.009), bloat (P=0.002), and swallowing difficulties (P=0.000) as the most critical clinical signs associated with increased mortality, with dysphagia emerging as the strongest predictor (Table 3).

Necropsy findings revealed extensive subcutaneous edema, particularly around the mandibular region. Hemorrhages were observed in the lungs, trachea, and heart, accompanied by severe congestion and frothy exudate. The nasal passages were occluded by thick, gummy mucus, and visceral organs exhibited congestion and petechial hemorrhages on serosal surfaces, indicative of widespread vascular damage and the highly fatal nature of the disease.

Histopathological findings

Histopathological examination of blood smears from infected buffaloes revealed numerous coccobacillary bacteria with characteristic bipolar staining. This staining pattern, observed under gram and Giemsa stains, confirmed the presence of *P. multocida*, consistent with septicemia.

Table 3. Observation and identification of significant clinical signs in buffaloes affected by HS

Clinical Signs	Status	No.		P
		Post-infection		
		Surviving Animals	Dead Animals	
Fever	Yes	25	18	0.231
	No	7	1	
Fluctuating fever	Yes	21	14	0.009*
	No	11	5	
Bloat	Yes	26	12	0.002*
	No	6	7	
Submandibular edema	Yes	26	13	0.325
	No	6	6	
Dyspnea	Yes	25	12	0.334
	No	7	7	
Dysphagia	Yes	27	13	0.000*
	No	5	6	

*Significant at 0.05 level.

Molecular detection using RT-PCR

Amplification plots generated from the qRTPCR assay (Figure 3) demonstrated characteristic exponential fluorescence curves for all qRTPCR-positive clinical samples, as well as for the positive control. Fluorescence signals became detectable after approximately 30 amplification cycles, suggesting a moderate abundance of the target gene in the samples tested. In contrast, no fluorescence signals were observed in the NTC reactions, indicating the absence of non-specific amplification and validating the assay's specificity.

These molecular findings conclusively confirmed the presence of *P. multocida* serotype B:2 in the tested blood and lung tissue samples. The results are consistent with the clinical signs and gross pathological lesions observed in the affected buffaloes, including respiratory distress, high fever, and hemorrhagic lesions. Although sequencing was not performed for further confirmation, the use of a validated primer-probe set and inclusion of controls support the specificity of the results. Future studies may incorporate genome sequencing to further validate strain identity and potential genetic variations.

Discussion

This study presented an epidemiological investigation of an HS outbreak in a water buffalo herd on Meghna River Island, Bhola, Bangladesh. The findings provide critical insights into disease dynamics, host susceptibility, and clinical outcomes in affected buffaloes, contributing to a deeper understanding of HS in endemic regions.

The outbreak investigation revealed a morbidity rate of 25.5% and a mortality rate of 9.5%, with a notably high case fatality rate of 37.3%. Calves, heifers, and adult female buffaloes exhibited greater susceptibility than males, aligning with previous research indicating high HS-related mortality in buffaloes (Almoheer et al., 2022; Habib et al., 2019). Similarly, an Indian study reported that buffaloes accounted for 86.53% of HS-related deaths, under-

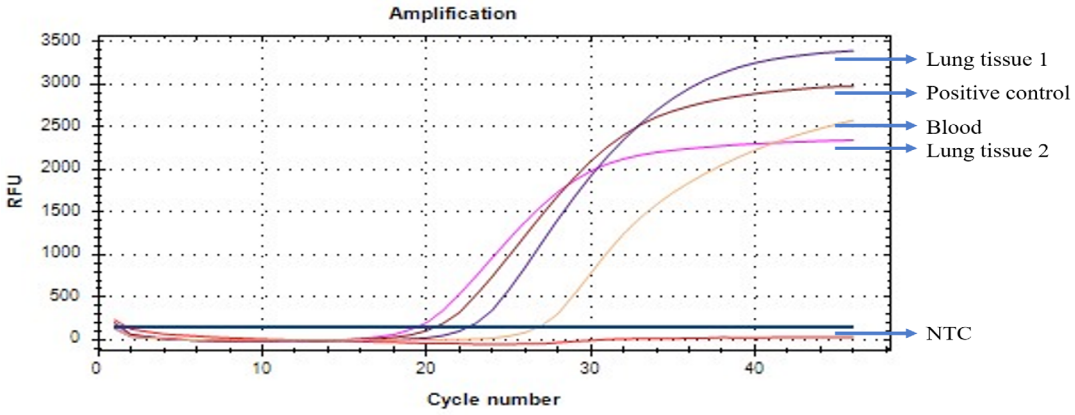

Figure 3. qRTPCR amplification curves for the detection of *P. multocida* serotype B:2 targeting the *Kmt1* gene

Note: Fluorescence (RFU) is plotted against cycle number for clinical samples, including two lung tissue samples (Cq: 22.60 and 19.46), one blood sample (Cq: 26.87), a positive control (vaccine strain, Cq: 20.58), and a NTC, which showed no amplification. All positive samples exhibited distinct exponential amplification phases, confirming the presence of *P. multocida* DNA. The absence of amplification in the NTC indicates no contamination or non-specific amplification. Reactions were performed using a TaqMan-based qRTPCR assay with LNA-modified probes, following thermal cycling parameters optimized for the *Kmt1* gene. Cq values were automatically calculated by the instrument software using a fluorescence threshold set during analysis (not shown). All reactions were run in single replicates. The axes are labeled according to standard qRTPCR output.

scoring their increased vulnerability (Mitra et al., 2013). However, a retrospective study in Bangladesh from 2010 to 2012 documented a case-fatality rate of 2.87% (Mondal & Yamage, 2014), suggesting that variations in outbreak severity, disease management strategies, or genetic factors may influence susceptibility.

The increased vulnerability of younger buffaloes, particularly calves, is consistent with findings that maternal immunity wanes after approximately 60 days of age, leaving unvaccinated calves highly susceptible (Mahmood et al., 2007). Delayed vaccination during this critical period further exacerbates infection risk and severe outcomes. Several studies have also demonstrated higher morbidity and mortality rates in buffaloes than in cattle during HS outbreaks. For example, a study in the Malakand district reported morbidity and mortality rates of 5.49% and 1.65%, respectively, in adult buffaloes, with a case fatality rate of 30% (Khan et al., 2006). This heightened susceptibility may be linked to genetic factors influencing immune response to *P. multocida*, the primary causative agent of HS (Ara et al., 2016). Understanding pathogen virulence and host immunity remains pivotal for developing effective disease control strategies (Shivachandra et al., 2011).

Environmental conditions in riverine regions, like Bhola, likely contributed to the persistence and transmission of *P. multocida*. The humid and wet climate in such areas provides a conducive environment for bacterial survival, increasing infection risks among water buffaloes. The outbreak, occurring on 19th March 2023 during the pre-monsoon season, may have been influenced by seasonal factors, such as high humidity, fluctuating temperatures, and poor husbandry practices (Almoheer et al., 2022; Cuevas et al., 2020). Heavy rainfall followed by hot weather may have led to immunosuppression, further facilitating disease spread. Additionally, the potential consumption of contaminated river water containing HS-infected carcasses could have played a role in transmission (Faez Firdaus et al., 2013).

The clinical presentation of fluctuating fever, bloat, and swallowing difficulties was strongly associated with increased mortality. These signs, documented as key indicators of severe HS infection, underscore the importance of early recognition for timely intervention (Shivachandra et al., 2011). Necropsy findings revealed hemorrhagic lesions in multiple organs, including the lungs and gastrointestinal tract, along with hallmark signs, such as hemorrhagic petechiae, subcutaneous edema, and lung consolidation (Arulmozhi et al., 2019).

The HS outbreak on Bhola Island underscores the urgent need for timely diagnosis, vaccination, and environmental management to prevent future outbreaks. Regular vaccination programs remain the most effective strategy for reducing HS incidence in endemic regions, particularly those prone to recurrent outbreaks (Shome et al., 2019; Zamri-Saad & Annas, 2016). Additionally, inadequate vaccination techniques and reduced vaccine efficacy may have contributed to the outbreak, emphasizing the necessity for improved immunization protocols.

Enhancing diagnostic capacities by establishing local laboratories capable of rapid *P. multocida* detection is essential for timely intervention. Early detection through molecular diagnostics can facilitate prompt treatment and containment of disease spread. Furthermore, implementing a comprehensive disease surveillance system would enable monitoring of livestock health, identification of early warning signs, and prevention of future outbreaks. Given the potential zoonotic transmission of *P. multocida*, a one-health approach integrating animal, environmental, and human health strategies is critical for effective HS control and public health protection.

Conclusion

This epidemiological investigation of an HS outbreak in a water buffalo herd on Meghna River Island, Bhola, Bangladesh, highlights the significant impact of the disease. The outbreak recorded an overall morbidity rate of 25.5%, a mortality rate of 9.5%, and a high case fatality rate of 37.3%, particularly in young and female buffaloes. Clinical signs, such as fluctuating fever and dysphagia, were strongly associated with increased mortality. The study emphasizes the importance of proper vaccination protocols, effective cold chain maintenance, and timely interventions to mitigate the spread of HS. Moreover, the potential zoonotic transmission of *P. multocida* from buffaloes to humans calls for a one-health approach to controlling HS for both animal and public health safety.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, the reliance on farmer-reported data introduces the potential for recall bias, particularly in the documentation of clinical signs and disease progression in affected buffaloes. Such biases may influence the accuracy of symptoms and outbreak chronology. Second, the study did not incorporate a systematic risk factor analysis, limiting the ability to quantify specific predisposing factors contributing to the outbreak. This constraint may have hindered a more comprehensive understanding of the epidemiological determinants of HS in the affected herd.

Despite these limitations, the study provides important epidemiological insights into HS in water buffaloes, particularly in a riverine ecosystem. The identification of key clinical signs, such as fluctuating fever and dysphagia, acting as strong predictors of mortality, highlights their importance in early disease detection and management. Future research should focus on integrating structured risk factor assessments and longitudinal surveillance to enhance outbreak characterization and disease control strategies.

Ethical Considerations

Compliance with ethical guidelines

This study does not require ethical approval, as it is conducted under the authority of the Field Disease Investigation Laboratory in Barishal, Bangladesh, under the Department of Livestock Services, Dhaka, Bangladesh, which is responsible for overseeing the research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization, supervision, methodology, and data analysis: Ibrahim Khalil; Sample collection: Shahin Mahmud and Khalilur Rahman; Diagnosis: Sajedul Hayat, Shukesh Chandra Badhy, and Md. Golam Azam Chowdhury; Data collection: Nafis Jawad; Writing the original draft: Ibrahim Khalil and Nafis Jawad; Review and editing: Abu Sayed, Tajul Islam Mamun, Sajedul Hayat, Shukesh Chandra Badhy, and Md. Nurul Alam; Supervision: Ibrahim Khalil, Abu Sayed, and Md. Golam Azam Chowdhury.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the valuable support provided by the local veterinary authority of Bhola Sadar in facilitating this investigation. The cooperation and contributions of the farmers are also deeply appreciated, as their assistance was essential to the successful completion of this study.

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