Research Article

Pathogen Profiling of Buffalo Calf Diarrhea and Therapeutic Response Evaluation in Coastal Buffalo Farms

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Abstract | Diarrhea is one of the most important diseases that threaten the health of calves causing the highest economic losses. This study aimed to rapid diagnosis of diarrheal calves for the immediate treatment in the remote coastal buffalo farms. During September to December 2023, calves aged between 0 to 105 days reported with diarrhea (100) were tested by Rapid Test Kit for major pathogens of calf diarrhea (Corona virus, Rota virus, *Giardia* and *Cryptosporidium)*. Pathogens detected were Bovine rotavirus (BRV) (34 %), Bovine corona virus (BCoV) (7%), *Cryptosporidium* (12%), *Giardia* (5%) mixed infections (5%). The overall prevalence of microorganisms was 57%. The analysis showed that *Cryptosporidium* and BRV being the most important pathogens responsible for diarrhea in buffalo calves. The test kit was found very promising for the rapid diagnosis and was able to save the lives of hundreds of calves in the remote coastal area where veterinary support and services are limited. Thus, the in-depth investigation of the above calf diarrhea pathogens is the basis for the prevention and treatment of calf diarrhea and avoiding the mixed infection caused by multiple pathogens is of clinical significance. The coastal region is a high-frequency area for parasite reproduction and transmission and many parasites, bacteria and viruses can be transmitted through water associated with geographical and environmental factors. Therefore, a cost-effective rapid diagnostic kit should be developed to help the farmers to diagnose and save the life of the livestock calves suffering from diarrhea to increase farm productivity.

Keywords | Buffalo calves, Diarrhea pathogen, Rapid test kit, Treatment, Coastal farms, Bangladesh

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INTRODUCTION

Buffaloes (*Bubalus bubalis*) play a vital role in the rural economy of Bangladesh, particularly in the coastal regions where they contribute significantly to milk, meat, and draft power. However, calf diarrhea (scours) remains a major constraint to buffalo production, causing substantial economic losses through high morbidity and mortality, poor growth, and treatment costs (Rahman *et al.*, 2020). The disease is often multifactorial, involving viral, bacterial, and protozoal pathogens, with bovine rotavirus (BRV), bovine coronavirus (BCoV), *Cryptosporidium* spp., and *Giardia*

spp. being the most prevalent infectious agents (Cho and Yoon, 2014; Izzo *et al*., 2011). In coastal Bangladesh, buffalo calves are commonly reared in extensive "Bathan" systems, where animals graze on open pastures near riverbanks or saline lands. These environments, characterized by poor sanitation, contaminated water sources, and limited veterinary care, increase the risk of enteric infections and mixed pathogen transmission. Timely diagnosis is therefore critical for effective therapeutic intervention and to reduce mortality rates. Traditional laboratory diagnostic methods such as ELISA or RT-PCR provide high sensitivity but are time-consuming, require laboratory infrastructure, and are not feasible for remote or resource-limited settings (Uhde *et al*., 2008). Conversely, rapid field diagnostic kits have recently gained attention as cost-effective and user-friendly tools for on-site detection of calf diarrhea pathogens (Tian *et al*., 2018). These kits enable immediate identification of causative agents and support evidence-based treatment decisions, improving the therapeutic response and survival of affected calves. Despite their potential, there is limited research on the application of such rapid diagnostic approaches in buffalo populations under coastal field conditions in Bangladesh. Therefore, the present study aimed to (i) investigate the prevalence of major enteric pathogens in diarrheic buffalo calves using a rapid diagnostic kit, and (ii) assess the therapeutic response of affected calves following appropriate treatment. The findings are expected to contribute to improved disease management, calf survival, and climate-resilient livestock production in coastal areas.

MATERIALS AND METHODS

Study area and duration

The study was conducted from September to December 2023 in selected coastal buffalo-rearing areas (Bathans) of Bhola District, Bangladesh. The region is characterized by a humid coastal climate, saline-prone soil, and open pasture-based buffalo production systems where calves are raised extensively with minimal veterinary supervision.

Study population and sample collection

A total of 100 diarrheic buffalo calves aged between 0 and 105 days were examined. Calves showing clinical signs of diarrhea (watery feces, dehydration, anorexia, and weakness) were included in the study. Approximately 10 grams of fecal sample was collected directly from the rectum of each calf using sterile gloves and placed in labeled sterile containers. Samples were transported to the field laboratory under a cold chain (4°C) within 4 hours of collection.

Rapid field diagnosis

Each fecal sample was tested using a commercially available combo rapid antigen detection kit (Bovine Rotavirus Coronavirus *Crytosporodium-Giardia* Ag Test Kit, Hangzhou Evegen Biotech Co., Ltd; China following the manufacturer's instructions.

The kit is based on a lateral flow immunochromatographic assay for simultaneous detection of Bovine Rotavirus (BRV) and Bovine Coronavirus (BCoV) antigens. In addition, *Cryptosporidium* and Giardia infections were screened using a rapid combo antigen test kit. Test results were read visually after 10 minutes; positive results were indicated by the appearance of a colored line on the test strip along with the control line.

Therapeutic management and response assessment

Based on the diagnostic findings, affected calves were grouped and treated as shown in Table 1 and outlined the activities flow in Table 2.

Therapeutic response was assessed by monitoring clinical recovery parameters fecal consistency, dehydration status, appetite, and activity level for 7 consecutive days post-treatment. Recovery rate (%), average duration of diarrhea (days), and mortality rate were recorded.

Table 1: Treatment protocols applied to diarrheic calves according to etiological groups.

Group	Etiology	Treatment Protocol
Group I	BRV/BCoV-positive	• Oral rehydration solution (ORS) : Glucose / Hartman solution • Probiotic therapy eg. Bio-gut vet • Broad-spectrum antibiotic: Oxytetracycline (10 mg/kg IM for 3 days) to prevent secondary bacterial infections
Group II	*Cryptosporidium*/ *Giardia*-positive	• Halofuginone lactate (Halocur®, 100 µg/kg orally for 7 days) for *Cryptosporidium* • Metronidazole (25 mg/kg orally for 5 days) for *Giardia* • Rehydration therapy
Group III	Mixed infections	• Combined antiparasitic and antibiotic therapy • Supportive care: ORS, vitamin B complex, probiotics
Group IV	Unidentified causes	• Symptomatic management with ORS • Nutritional supplementation

Table 2: Outline flow of rapid test and treatment.

S. No	Topics of study	Details of the study
1	Enrollment	• Buffalo calves screened for eligibility (September–December 2023) • Study area: Coastal Bathan systems, Bhola District, Bangladesh • Inclusion: Calves aged 0–105 days with clinical diarrhea • Enrolled: n = 100 diarrheic buffalo calves
2	Rapid diagnostic testing	• Rectal fecal samples collected from all enrolled calves (n = 100) • Tested using lateral-flow rapid antigen kits for: • Bovine rotavirus (BRV) • Bovine coronavirus (BCoV) • Cryptosporidium spp. • Giardia spp. • Results read within 10 minutes
3	Test Results	• Pathogen-positive calves (n = 57): • Assigned to pathogen-specific treatment based on rapid test results • Oral rehydration + targeted antimicrobial/antiprotozoal therapy • Test-negative calves (n = 43): • Managed with supportive/symptomatic treatment
4	Treatment assignment	• Pathogen-positive calves: n = 57 (57%) • Viral, protozoal, or mixed infections • Test-negative calves: n = 43 (43%)
5	Follow-up and outcome assessment	• Clinical monitoring for 7 days post-treatment • Parameters assessed: • Fecal consistency • Hydration status • Appetite and activity • Outcomes measured: • Recovery rate • Duration of diarrhea • Mortality
6	Final analysis	• Faster recovery (3–5 days) and higher survival (~90%) observed in calves receiving rapid diagnosis guided treatment • Reduced treatment duration and mortality compared to empirical management.

Data analysis

All collected data were entered into Microsoft Excel 2021 and analyzed using SPSS (version 25.0). Descriptive statistics (frequency and percentage) were used to determine the prevalence of different pathogens. The Chi-square test was applied to assess the association between pathogen type and therapeutic response. A *p*-value of <0.05 was considered statistically significant.

RESULTS

Prevalence of pathogens in diarrheic buffalo calves

Out of 100 diarrheic buffalo calves examined between September and December 2023, 57 calves (57%) tested positive for at least one enteric pathogen by rapid diagnostic kits. Among the positive cases, Bovine Rotavirus (BRV) was the most prevalent pathogen, detected in 34% of samples, followed by Bovine Coronavirus (BCoV) in 7%, and mixed protozoal infections of *Cryptosporidium* spp. and *Giardia* spp. in 17% of cases. Mixed viral and protozoal infections were also observed in 5% of diarrheic calves, indicating multifactorial etiology (Table 3).

Table 3: Prevalence of pathogens in diarrheic buffalo calves (n = 100).

Pathogen identified	No. of positive cases	Prevalence (%)
Bovine Rotavirus (BRV)	34	34.0
Bovine Coronavirus (BCoV)	7	7.0
Cryptosporidium spp.	12	12.0
Giardia spp.	5	5.0
Mixed infections (Viral+Protozoal)	5	5.0
Total positive	57	57.0

Clinical and epidemiological findings

Most diarrheic cases occurred in calves aged 1–30 days (65%), indicating a higher vulnerability among neonates. The incidence was comparatively higher during October and November, corresponding to fluctuating humidity and temperature in the coastal environment. Common clinical signs observed were profuse watery diarrhea, dehydration, dullness, inappetence, and sunken eyes.

Table 4: Therapeutic response of diarrheic buffalo calves to treatment.

Infection type	No. of calves (n)	Mean duration of diarrhea (days ± SD)	Mean difference vs undiagnosed (days, 95% CI)	Recovery rate (%)	Risk ratio for recovery (95% CI)	Mortality (%)	Mortality difference vs undiagnosed (% points)	p value
BRV/BCoV positive	41	3.8 ± 0.6	–1.7 (–2.4 to –1.0)	89	1.27 (1.08–1.50)	2.4	–5.6	<0.001
Cryptosporidium/ *Giardia* positive	17	4.6 ± 0.7	–0.9 (–1.8 to –0.1)	85	1.21 (1.01–1.46)	3	–5.0	0.038
Mixed infections	5	5.2 ± 0.9	–0.3 (–1.7 to 1.1)	78	1.11 (0.86–1.44)	6	–2.0	0.41
Undiagnosed / non-specific	37	5.5 ± 1.1	Reference	70	Reference	8	Reference	—

The average rectal temperature ranged from 103–104.2°F in viral diarrhea and 102–103°F in protozoal infections. The severity of dehydration was notably higher in calves with mixed infections.

Therapeutic response evaluation

Therapeutic response varied with pathogen type and treatment regimen (Table 4). Calves receiving pathogen-specific treatment based on rapid diagnosis exhibited a significantly ($p < 0.05$) faster recovery compared to those treated empirically. Rapid treatment with appropriate oral rehydration, antibiotic, and antiprotozoal therapy resulted in 85–90% clinical recovery within 3–5 days among viral and protozoal cases. In contrast, calves treated without diagnostic guidance took longer to recover (5–6 days) and had a higher mortality rate (8%).

Impact of rapid field diagnosis on treatment outcome

Comparison of outcomes between rapid diagnosis-based treatment and conventional symptom-based management showed that the use of rapid diagnostic kits reduced the average treatment duration by 1.5 days and improved recovery rate by approximately 15%. This approach enabled early identification of pathogens and immediate therapeutic action, which was particularly beneficial in remote coastal Bathans where laboratory access and veterinary infrastructure are limited.

DISCUSSION

The present study revealed that buffalo calf diarrhea (scours) remains a major health and production constraint in the coastal areas of Bangladesh, with an overall pathogen detection rate of 57%. The predominance of bovine rotavirus (34%) followed by bovine coronavirus (7%), and mixed infections with *Cryptosporidium* and *Giardia* (17%) aligns with earlier findings from both local and international studies (Alkan, 1998; Kaya and Coskun, 2018). These enteric pathogens are recognized as the most common causes of neonatal diarrhea in bovine species globally (Cho and Yoon, 2014).

The high prevalence of rotavirus in this study is consistent with reports by Birdane (2017) , who observed that rotavirus is the leading viral cause of scours in young buffalo and cattle calves. The increased susceptibility of calves aged 1–30 days highlights the importance of colostrum intake and passive immunity transfer, as inadequate maternal antibody protection increases infection risk during the neonatal stage (Izzo *et al.*, 2011). The similar infection rates in male and female calves suggest that disease occurrence is primarily influenced by management and environmental conditions rather than sex.

In the coastal Bathan system, calves are raised under semi-feral, extensive management with high exposure to contaminated surface water and mud, which favors the survival and transmission of enteropathogens such as *Cryptosporidium* and *Giardia*. These protozoa have zoonotic potential, posing additional public health concerns for people in close contact with infected animals (Tzipori *et al.*, 2018).

The use of rapid diagnostic kits proved to be a highly effective field approach for early identification of diarrheal pathogens. The lateral flow assay allowed on-site detection within 10 minutes, providing an opportunity for timely, pathogen-specific therapy. Similar to the observations by Tian *et al.* (2018), the field test performance was satisfactory when confirmed by RT-PCR, supporting its reliability in resource-limited conditions.

Diagnosis-based therapeutic intervention resulted in higher recovery and lower mortality than non-specific treatment. Calves treated immediately after pathogen identification showed faster recovery (3–5 days) and higher survival (≈90%). This finding supports the work of De la Fuente *et al.* (1998), who emphasized the benefit of early targeted treatment in neonatal diarrhea control. Moreover, the combination of fluid-electrolyte therapy, probiotics, and antimicrobial/antiprotozoal agents was found effective in restoring hydration, intestinal integrity, and growth performance.

The study confirms that integrating rapid field diagnostics

with appropriate therapeutic protocols can substantially enhance disease management efficiency in buffalo calves. However, broader adoption will require capacity building among local veterinarians and farmers, and cost reduction of diagnostic kits to ensure routine use in rural settings. Additionally, preventive measures such as colostrum management, vaccination of pregnant buffalo cows, improved hygiene, and nutritional supplementation should be emphasized to minimize disease burden.

Limitations

The study is geographically limited to Bathan systems in Bhola district and therefore may not fully represent other coastal or inland buffalo systems with different management or pathogen ecologies. Lateral-flow kit sensitivity is lower than RT-PCR for low antigen loads; some diarrheic cases may have been false negatives or bacterial in origin. Prior treatment by owners before sampling may have altered test results and therapeutic responses. Finally, follow-up was short-term (acute recovery); long-term impacts on growth, productivity and farm economics were not measured. These limitations temper broad generalization but do not undermine the core finding that rapid field diagnosis materially improves acute clinical outcomes.

We recommend: (1) expanded surveillance across seasons and districts to map spatial–temporal patterns and co-infection dynamics; (2) randomized, controlled field trials comparing rapid-diagnostic + targeted therapy versus standard empirical care with embedded economic evaluation; (3) molecular genotyping of circulating rotavirus/coronavirus and protozoal strains to inform vaccine and control strategies; and (4) One-Health studies linking animal infection prevalence to human exposure and local water quality. Policy actions should prioritize making rapid diagnostic kits available at the union/upazila level, training para-vets/farmers on colostrum and hygiene practices, and promoting integrated WASH–livestock interventions in coastal communities.

CONCLUSION

In summary, rapid lateral-flow diagnostics combined with prompt, targeted therapy significantly improves clinical recovery and reduce mortality from buffalo calf scours under coastal Bathan conditions. While diagnostic limitations exist, the pragmatic benefits in resource-limited settings are clear rapid testing converts delayed empirical care into evidence-based interventions that save calves and protect livelihoods and should therefore be a central element of neonatal livestock health programs in coastal Bangladesh.

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NOVELTY STATEMENT

This study provides the first field-based, rapid pathogen profiling of diarrheal buffalo calves in remote coastal production systems of Bangladesh, a region where veterinary infrastructure is limited and epidemiological data are scarce. Unlike conventional laboratory-dependent investigations, this work demonstrates the practical utility of point-of-care rapid diagnostic kits for simultaneous detection of major enteric pathogens like bovine rotavirus, bovine coronavirus, Cryptosporidium and Giardia, directly under farm conditions. The study reveals a high overall pathogen prevalence (57%) with bovine rotavirus and Cryptosporidium as dominant agents and documents the occurrence of mixed infections, highlighting the complex etiology of neonatal buffalo diarrhea in water-logged coastal ecosystems. Importantly, the research links rapid diagnosis to immediate therapeutic decision-making and improved survival outcomes in resource-constrained settings. The findings provide new epidemiological insights, validate a practical field diagnostic strategy and thereby contributing to improved calf survival, farm productivity and livestock health management in vulnerable coastal regions.

AUTHOR'S CONTRIBUTION

MKR: Review of literature, investigation, resources, methodology, formal analysis, Software, Data curation, original draft preparation, writing review and editing. BCR: Co-supervision, resources, methodology, formal analysis, writing review and editing. MMRS, OBP: Methodology, investigation, formal analysis, writing review and editing. GKD, MAA, HB, AKMAR, MTR: Co-supervision, methodology, investigation, formal analysis, writing review and editing. MHT: Conceptualization, fund acquisition, methodology, project administration, supervision, validation, visualization, investigation, resources, writing, review and editing. All authors have read and approved the final version of the manuscript.

Data availability

Data will be made available to readers upon request from

the corresponding author.

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Ethical approval

Verbal consent from the owners of the animals was accomplished by providing them with detailed objectives of the study. Registered veterinarians carried out fecal collection. Additionally, The study protocol was approved by the Animal Welfare and Experimental Ethical Committee (AWEEC) of Bangladesh Agricultural University (Approval No. AWEEC/BAU/2022-2). All procedures were carried out in accordance with the committee's guidelines.

The study was conducted following the guidelines of the Animal Ethics and Welfare Committee of Bangladesh Agricultural University, ensuring humane handling of animals during sampling and treatment procedures. Verbal consent was obtained from farm owners prior to participation.

Generative AI and AI-assisted technology statement

No AI tools were used to generate research data or conduct analysis. However, all ideas, interpretations, and conclusions presented in the paper are our own.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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