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Research Article

Prevalence and Diversity of Gastrointestinal Parasites Associated with Diarrhea in Buffalo Calves Under Extensive Buffalo Farming in Bangladesh

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Abstract | Buffaloes (*Bubalus bubalis*) are vital to Bangladesh's livestock economy, contributing significantly to milk, meat, and draught power. Despite their importance, buffalo health, especially among calves, remains under-researched, particularly gastrointestinal (GI) parasitism, a major contributor to calf morbidity and mortality. This study aimed to assess the prevalence, diversity and associated risk factors of GI parasites in diarrheic buffalo calves across three ecologically distinct regions such as Bhola (coastal), Rangpur (wetland), and Jamalpur (riverbank) in Bangladesh. A total of 625 diarrheic fecal samples were collected from buffalo calves aged 1–6 months during January 2022 to December 2024 and examined using direct smear, flotation, and sedimentation techniques. Overall, 57.28% of calves were infected with one or more GI parasites. Helminths identified included *Toxocara vitulorum* (24.8%), *Capillaria* spp. (10.72%), *Strongyloides* spp. (6.72%), *Trichuris* spp. (5.12%), *Fasciola gigantica* (8.96%), *Amphistomes* (14.08%), and *Moniezia* spp. (5.12%). Protozoa detected were *Balantidium coli* (16.48%), *Eimeria* spp. (8.64%), and *Cryptosporidium* spp. (7.04%). Age significantly influenced parasite prevalence, with higher infection rates observed in 1–3-month-old calves (69.2%) than those aged 3–6 months (34.27%). No significant sex-based differences were observed, though males tended to harbor higher parasite loads. Co-infections were common, with up to six parasite species detected in some calves. Poor housing, lack of deworming, shared grazing lands and contaminated water sources were identified as key risk factors for occurrence of GI parasites in buffalo calves. The study underscores the urgent need for targeted parasite control strategies, particularly in young calves, and improved husbandry practices to mitigate the economic losses are associated with GI parasitism in buffalo farming systems.

Keywords | Buffalo calves, Diarrhea, GI parasites, Prevalence, Risk factors, Bangladesh

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INTRODUCTION

The domestic water buffalo (*Bubalus bubalis*) is a vital ruminant species reared primarily for milk, meat, and draught power across many parts of the world. In Bangladesh, buffaloes are considered second to cattle in terms of economic contribution, playing a significant role in the livelihoods of smallholder farmers and in the national livestock economy (Sarker *et al*., 2013). Although the buffalo population is approximately 1.5 million, the species holds a disproportionately important place in the country's livestock sector. Buffaloes in Bangladesh are predominantly raised under the extensive *Bathan* farming system, particularly in coastal areas, and smallholder household-level farming is also common. Among domestic livestock, buffaloes are especially well-suited to the tropical and subtropical climates of countries like Bangladesh (Das and Khan, 2010). Known as "triplet animals" due to their provision of milk, meat, and draught power, they are considered highly valuable (Ghaffar *et al*., 1991). However, buffalo productivity is often hampered by poor breeding and feeding practices, as well as by a wide range of infectious diseases, most notably, parasitic infections. Parasitic infestations, particularly those of the gastrointestinal (GI) tract, are a major constraint in livestock production in Bangladesh. The warm and humid climate of the country provides ideal conditions for the survival, development, and transmission of various parasites. Parasitism can result in significant economic losses through mortality, reduced productivity, poor growth, lowered fertility, decreased milk and meat yield, increased treatment costs, and involuntary culling (Faiz, 1972; Raza *et al*., 2007). Gastrointestinal parasitism in buffaloes is of particular concern, as it negatively affects animal health and growth, especially in calves. The severity of infection and its effects may vary with factors such as age, sex, nutritional status, and parasite load. Helminth infections, including those caused by *Strongyle*, *Toxocara*, and protozoan parasites like *Eimeria* and *Cryptosporidium*, have been reported to cause both clinical and subclinical disease in calves, sometimes leading to fatal outcomes (Gaddam, 2005; Abdel-Rahman *et al*., 2013; Olias *et al*., 2011). In neighboring countries such as Pakistan, the prevalence of GI helminths in ruminants has been reported to range from 25% to 92%, depending on geographic region and study period (Raza *et al*., 2007; Kakar *et al*., 2008). In Bangladesh, some studies have reported the prevalence of GI parasitism in various districts (Alim *et al*., 2000; Saha *et al*., 2013; Biswas *et al*., 2014; Roy *et al*., 2011; Rahman *et al*., 2017). However, in-depth studies focusing specifically on diarrheic buffalo calves remain limited.

Fecal examination remains the primary diagnostic method for GI parasitism, often involving techniques such as fecal flotation, sedimentation, and culture, along with clinical assessment. As buffalo calves represent the future of the livestock industry, ensuring their health particularly through early diagnosis and control of parasitic infections is essential for sustainable and profitable buffalo farming. Given the growing interest in buffalo farming in Bangladesh and the government's emphasis on its development into a commercial sector, there is an urgent need to address calf health issues, especially diarrhea. Diarrhea in calves is often associated with parasitic infections, yet there is limited comprehensive research on the specific GI parasites responsible. Therefore, the present study aimed to identify and characterize gastrointestinal parasites associated with diarrhea in buffalo calves, with a focus on variations related to age, sex, and geographical location across selected buffalo farms in Bangladesh.

MATERIALS AND METHODS

Study area and sampling strategy

The study was conducted among diarrheic calves on selected buffalo farms located in the Bhola, Rangpur, and Jamalpur districts of Bangladesh (Figure 1). Bhola district, the largest island in the southern coastal region of Bangladesh, has the highest concentration of buffalo farms. Rangpur district, specifically Gangachara Upazila, features agricultural and riverine landscapes with extensive wetlands and the Teesta–Ghaghat river system. Jamalpur district, particularly Madarganj Upazila, is a notable buffalo-farming region situated along the banks of the Jamuna River and plays a significant role in local livelihoods and buffalo husbandry. All laboratory analyses were performed at the Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202.

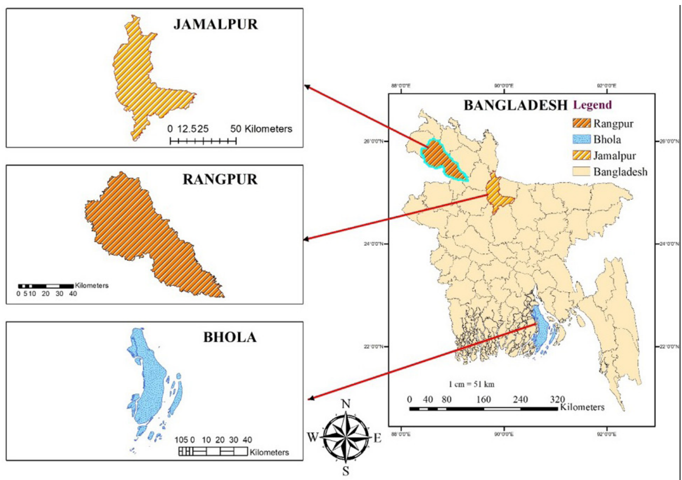

Figure 1: Map of Bangladesh showing the districts of buffalo farms of the study.

Sample collection and microscopic examination

A team of field veterinarians in each of the three study areas was responsible for identifying cases of diarrhea in buffalo calves and collecting fecal samples from affected animals from January 2022 to December 2024 (Figure 2). A total of 625 fresh fecal samples were collected from diarrheic buffalo calves. Approximately 10 grams fecal sample was collected immediately following the onset of diarrhea, placed in a labeled and numbered zipper bag, and stored under cool conditions during transportation to the laboratory. The samples were delivered to the Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh for coproscopic examination. Each sample was processed using the direct wet mount, sedimentation technique (as described by Yasin *et al*., 2018), and flotation and McMaster methods (as described by Soulsby, 1987) and eggs, cysts, and oocysts of helminths and protozoa were identified under microscope. Parasites were identified based on morphological characteristics using standard identification keys and descriptions provided by Soulsby (1987) and Thienpont *et al*. (1986).

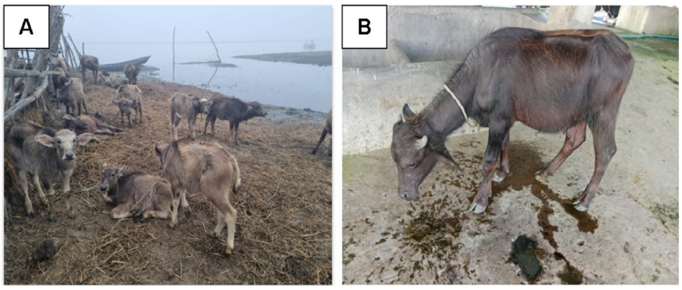

Figure 2: Rearing systems and common diseases of buffalo calves. (A) Buffalo calves rearing in free range area in coastal area of Bangladesh; (B) Diarrheic buffalo calf.

Statistical analysis

Data were analyzed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA) to evaluate the statistical significance and intensity of associations between gastrointestinal (GI) parasite infections and various predictor variables. Initially, univariate analysis was performed to identify individual risk factors associated with GI parasite infection. Factors showing significant associations ($p < 0.05$) in the univariate analysis were selected for multivariable analysis using multiple logistic regression with backward stepwise elimination. In the final model, variables were retained or excluded based on p-values of 0.05 and 0.1, respectively. A p-value of <0.05 was considered statistically significant.

RESULTS

Overall prevalence of GI parasites in buffalo calves

During the study period, a total of 625 buffalo calves affected by diarrhea were examined using coproscopy. Among them, 358 calves were found to be infected with one or more species of gastrointestinal (GI) parasites, indicating an overall prevalence of 57.28%. Morphological identification of eggs, cysts, and oocysts revealed the presence of parasites from three classes of helminths (Trematoda, Cestoda, and Nematoda) as well as three genera of protozoa.

Among the helminths, the prevalence rates were as follows, Nematodes *Toxocara* spp. (24.8%), *Trichuris* spp. (5.12%), *Strongyloides* spp. (6.72%), and *Capillaria* spp. (10.72%), Trematodes, Amphistomes (14.08%) and *Fasciola gigantica* (8.96%), Cestodes: *Moniezia* spp. (5.12%), among the protozoan parasites, the following prevalence rates were recorded, *Cryptosporidium* spp. (7.04%), *Eimeria* spp. (8.64%), *Balantidium coli* (16.48%) (Table 1). The findings indicate that *Toxocara* spp. had the highest prevalence among the helminth infections. Among the protozoan infections, *Balantidium coli* was the most prevalent, followed by *Eimeria* spp. and *Cryptosporidium* spp.

Table 1: Gastrointestinal parasites detected in the diarrheal feces of buffalo calves population in selected buffalo farms in Bangladesh.

Types of parasites	Genera of parasites	No of infected/ No. of examined	Prevalence %	Confidence level 95% (Range)	EPG Mean+SE
Nematode	*Toxocara vitulorum*	155/625	24.8	12.19 (35.20-60.20)	48.01±6.20
	Trichuris spp.	32/625	5.12	9.67 (6.97-26.33)	16.65±4.92
	Strongyloides spp.	42/625	6.72	7.95 (3.38-19.28)	11.33±4.04
	Capillaria spp.	67/625	10.72	4.14 (11.91-19.64)	15.49±2.11
Trematode	*Fasciola* sp.	56/625	8.96	15.48 (12.68-28.14)	28.14±7.87
	Amphistomes	88/625	14.08	9.30 (18.10-36.82)	27.46±4.76
Cestode	*Moniezia* spp.	32/625	5.12	5.01 (6.41-16.44)	11.42±2.24
Protozoa	*Eimeria* spp.	54/625	8.64	6.76 (10.92-24.45)	35.38±17.96
	Giardia. spp.	51/625	8.16	8.63 (9.92-27.20)	18.55±4.39
	Cryptosporidium sp.	44/625	7.04	10.63 (9.08-29.55)	33.19±10.53
	Blantidium coli	103/625	16.48	11.12 (23.12-45.36)	34.24±5.56
	Total	358/625	57.28		
	Total Protozoal infection	252/625	40.32		
	Total Helminths infection	472/625	75.52		

n=Total animals examined; CI= Confident interval.

Co-infection rates of GI parasites in buffalo calves

Based on the data the results regarding co-infections of parasites among buffalo calves are summarized in Table 2. Out of a total of 625 buffalo calves examined, 358 calves (57.28%) were found to be infected with one or more types of parasitic infections. Single infections were the most common, observed in 191 calves, accounting for 30.56% of the total examined population. Double infections were recorded in 67 calves (10.72%). Triple infections affected 43 calves (6.88%), Quadruple infections were identified in 29 calves (4.64%). Pentacle infections (i.e., infections with five different parasites) were seen in 17 calves (2.72%), Hexacle infections (infections involving six different parasites) were the least common, found in 11 calves (1.76%). These results indicate that while single infections were predominant, a significant proportion of the calves also suffered from multiple (co-)infections, highlighting the complexity and severity of parasitic infestation in the studied population.

Table 2: Co-infection rates of Gastrointestinal Parasites in buffalo calves.

S. No.	Types of infections	No of infected/ No. of examined	Prevalence %
1	Single	191/625	30.56
2	Double	67/625	10.72
3	Triple	43/625	6.88
4	Quadruple	29/625	4.64
5	Pentacle	17/625	2.72
6	Hexacle	11/625	1.76
	Total	358/625	57.28

Age-wise prevalence of GI parasites in buffalo calves

The prevalence of GI parasites in buffalo calves showed significant variation between two age groups: 1–3 months and 3–6 months. Among the 1–3 month-old calves (n = 412), a total of 285 calves (69.2%) were found to be infected with one or more GI parasites. In contrast, the 3–6 month-old calves (n = 213) had a significantly lower infection rate of 34.27% (73/213). In both age groups, *Toxocara vitulorum* was the most prevalent parasite, with slightly higher infection in older calves (26.76%) than in younger ones (23.8%). *Balantidium coli* was the most common protozoan parasite, showing a similar prevalence in both age groups (16.5% in 1–3 months vs. 16.43% in 3–6 months). *Amphistomes* also had comparable prevalence across both groups (14.1% and 14.08%, respectively), though the odds of infection in the 1–3 month group were significantly higher (OR = 2.02, p = 0.0003).

Younger calves (1–3 months) generally exhibited higher prevalence for most parasites, including *Strongyloides* spp., *Capillaria* spp., *Trichuris* spp., *Fasciola* spp., and *Eimeria* spp., indicating greater susceptibility at an early age. Additionally, the egg per gram (EPG) counts tended to be higher in the younger group for several parasites, highlighting increased parasite load and infection intensity in early life. These findings underscore the importance of early-age deworming and targeted parasite control strategies in young buffalo calves to mitigate GI parasitic burden.

Sex-wise prevalence of GI parasitic infections in buffalo calves

The study revealed minimal sex-based variation in the prevalence of gastrointestinal (GI) parasites among buffalo calves. Among male calves (n = 356), 203 (57.02%) were infected with one or more GI parasites, while among female calves (n = 269), 149 (55.39%) were infected. The odds ratio (OR = 1.029; 95% CI: 0.71–1.34; p < 0.001) indicated no statistically significant association between sex and overall GI parasite infection.

In both sexes, *Eimeria* spp. was the most prevalent parasite, affecting 28.65% of males and 19.70% of females, with males also showing a higher average oocyst load (33.48 ± 9.67 EPG) compared to females (10.50 ± 2.12 EPG). Similarly, *Balantidium coli* was commonly found in both groups, with a prevalence of 17.42% in males and 15.24% in females. Notably, *Trichuris* spp. had a considerably higher prevalence in males (13.76%) than in females (6.69%), while *Capillaria* spp. showed an opposite trend, being more prevalent in females (18.77 ± 7.59 EPG) despite a lower infection rate (2.60%).

In terms of infection intensity (EPG), males generally exhibited higher egg or cyst loads for most parasites, including *Giardia* spp. (67.5 ± 56.3), *Moniezia* spp. (39.5 ± 28.3), and *Cryptosporidium* spp. (17.13 ± 8.51), compared to females. Although some individual parasite prevalences differed slightly between sexes, these differences were not statistically significant. Overall, the results suggest that sex is not a major determining factor in GI parasitic infection rates in buffalo calves, although males may experience higher infection intensities in certain cases.

DISCUSSION

Despite the significant role of buffaloes in the rural economy of Bangladesh particularly in milk and draught power production, this species has historically remained under-researched and underutilized (Habib *et al*., 2017). The current study represents one of the first systematic investigations in Bangladesh that evaluates the prevalence and diversity of gastrointestinal (GI) parasites in diarrheic buffalo calves across different districts in Bangladesh, with emphasis on co-infection patterns, regional, age-wise, and sex-wise variations. The overall prevalence of GI parasites in diarrheic calves was 57.28%, reflecting a substantial parasitic burden within this population. The use of both direct smear and concentration techniques likely enhanced the sensitivity of parasite detection.

This finding is comparable with previous studies in tropical and subtropical regions, where GI parasitic infections in young ruminants are a significant cause of morbidity and cause huge production loss (Singh *et al*., 2006; Khurana and Sethi, 2017).

Table 3: Regional prevalence of GI parasites of diarrheal buffalo calves.

Region	Name of parasites	No. of infected/ No. examined	Prevalence % and 95% CL Range	EPG Mean± SE	Odds ratio	p value
Bhola district (n=371)	*Amphistomes*	51/371	13.7 (11.76-25.18)	28.61±10.83	1.18	.0003*
	Toxocara vitulorum	107/371	28.8 (18.51-29.65)	33.19±10.44		
	Strongyloides sp.	29/371	7.8(9.66-18.76)	51.52±20.77		
	Capillaria sp.	44/371	11.9(1.48-11.56)	13.20±3.29		
	Trichuris sp.	22/371	5.9 (1.23-16.24)	11.49±2.11		
	Fasciola sp.	39/371	10.5(1.45-6.46)	9.20±1.19		
	Moniezia sp.	21/371	5.7(5.18-12.10)	13.86±4.75		
	Eimeria sp.	39/371	10.52(14.76-25.18)	36.67±7.81		
	Giardia sp	35/371	8.63(4.92-27.20)	11.55±3.39		
	Cryptosporidium sp.	29/371	7.81(4.62-16.72)	27.68±11.82		
	Balantidium coli	68/371	18.3(21.20-36.80)	47.67±.8.12		
	Sub Total	244/371	65.8			
Rangpur district (Gangachara, n= 110)	*Amphistomes*	14/110	12.73 (14.76-25.18)	28.61±10.83		0.15
	Toxocara vitulorum	18/110	16.36(18.51-29.65)	33.19±10.44		
	Strongyloides sp.	5/110	4.55(9.67-18.76)	51.52±20.77		
	Capillaria sp.	10/110	9.09(17.78-31.56)	13.20±3.29		
	Trichuris sp.	4/110	3.64 (6.42-18.22)	17.4±4.75		
	Fasciola sp.	1/110	0.910(1.02-11.37)	9.20±1.19		
	Moniezia sp.	5/110	4.55(4.18-11.10)	13.86±4.75		
	Eimeria sp.	7/110	6.36(14.76-25.18)	36.67±7.81		
	Giardia sp	7/110	6.36(3.42-21.57)	7.59±11.75		
	Cryptosporidium sp.	6/110	5.45(4.62-16.72)	27.68±11.82		
	Balantidium coli	12/110	10.91(19.20-36.80)	47.67±.8.12		
	Sub Total	47/110	42.73			
Jamalpur (Madargonj, n= 154)	*Amphistomes*	23/154	14.94 (14.76-25.18)	28.61±10.83		0.33
	Toxocara vitulorum	30/154	19.48 (13.67-21.53)	33.19±10.44		
	Strongyloides sp.	8/154	5.19(9.66-18.76)	51.52±20.77		
	Capillaria sp.	13/154	8.44(7.78-17.56)	13.20±3.29		
	Trichuris sp.	6/154	3.9 (7.11-21.31)	14.4±4.75		
	Fasciola sp.	16/154	10.39(7.4- 14.75)	9.20±1.19		
	Moniezia sp.	6/154	3.90(4.18-11.10)	13.86±4.75		
	Eimeria sp.	8/154	5.19(11.76-25.18)	36.67±7.81		
	Giardia sp	9/154	5.84(4.47-12.82)	7.41±12.75		
	Cryptosporidium sp.	9/154	5.84(14.62-26.72)	27.68±11.82		
	Balantidium coli	23/154	14.94(17.20-36.80)	47.67±.8.12		
	Sub Total	67/154	43.51			

Table 4: Prevalence of GI parasites of buffalo calves associated with age.

Parameter	Name of parasites	No. of animal Infected/ examined	Prevalence % And CL(Range)	EPG Mean± SE	Odds Ratio	p value
Calves (1-3 Months)n=412	*Amphistomes*	58/412	14.1 (11.1-22.04)	27.44 ± 5.52	2.02	0.0003*
	Toxocara vitulorum	98/412	23.8 (13.88-33.98)	11.16 ± 4.62		
	Strongyloides sp.	32/412	7.8 (2.56-9.59)	7.15 ± 2.64		
	Capillaria sp.	48/412	11.7 (9.56-19.59)	20.9 ± 11.0		
	Trichuris sp.	24/412	5.8(4.05-4.37)	16.57 ± 0.837		
	Fasciola sp.	46/412	11.2(11.03-26.70)	9.77 ± 4.67		
	Moniezia sp.	28/412	6.8(4.66-13.01)	176.6 ± 93.8		
	Eimeria sp.	43/412	10.4(9.68-20.16)	26.49 ± 6.85		
	Giardia sp	41/412	10.0 (7.78-18.17)	12.02 ± 5.86		
	Cryptosporidium sp.	32/412	7.8(2.97-10.29)	13.26 ± 6.20		
	Balantidium coli	68/412	16.5(12.16-18.89)	8.10 ± 2.96		
	Sub Total	285/412	69.2			0.15
Calves(3-6 Months) n=213	Amphistomes	30/213	14.08 (7.16-7.25)	14.34 ± 7.39		
	Toxocara vitulorum	57/213	26.76 (4.13-11.18)	19.1 ± 10.7		
	Strongyloides sp.	10/213	4.69(0.55-4.85)	13.51 ± 7.23		
	Capillaria sp.	19/213	8.92 (1.13-6.07)	21.14 ± 9.74		
	Trichuris sp.	8/213	3.76 (2.41-8.40)	6.00 ± 2.07		
	Fasciola sp.	10/213	4.69(3.04-7.57)	4.80 ± 2.86		
	Moniezia sp.	4/213	1.88 (1.76-7.25)	97.0 ± 63.2		
	Eimeria sp.	11/213	5.16(5.21-12.81)	18.73 ± 7.40		
	Giardia sp	10/213	4.69(0.84-5.47)	97.1 ± 90.2		
	Cryptosporidium sp.	12/213	5.63 (1.13-6.07)	21.1 ± 10.7		
	Balantidium coli	35/213	16.43 (3.43-10.08)	15.68 ± 6.10		
	Sub Total	73/213	34.27			

The findings reveal a high overall prevalence (57.28%), which is in agreement with previous reports from India (54.12–70.45%) (Zhang *et al*., 2020) and higher rates reported in Italy (5.4–33.1%) (Condoleo *et al*., 2007), in Pakistan (29.04%) and in Mexico (32.6–54.6%). Particularly noteworthy is the 100% prevalence in coastal areas, indicating a critical regional hotspot for GI parasitism in buffalo calves, which is consistent with earlier reports from certain regions of Bangladesh and higher than that reported previously from Greece (92.7%), Nepal (34.4–86%) and Poland (44%) (Biswas *et al*., 2021; Lamsal *et al*., 2020). Among helminth infections, *Toxocara vitulorum* was the most prevalent (24.8%), consistent with reports from India, Pakistan, and Nepal, where *T. vitulorum* is considered a common and highly pathogenic nematode in buffalo calves under six months of age (Roberts *et al*., 2024; Wadhwa *et al*., 2011). Its high prevalence in the current study can be attributed to transcolostral transmission, a well-documented route in buffalo calves (Dorny *et al*., 2015).

Among protozoan parasites, *Balantidium coli* was the most frequently identified (16.48%), followed by *Eimeria* spp. (8.64%) and *Cryptosporidium* spp. (7.04%). The detection of *B. coli* in a high proportion of calves, especially those under three months of age, aligns with earlier findings in water buffaloes and pigs in Southeast Asia (da Silva Barbosa *et al*., 2015), and may be linked to poor hygiene and close contact with contaminated water sources. *Eimeria* and *Cryptosporidium* spp. are common enteropathogens in young calves and can contribute to significant diarrheal illness and growth retardation (Fayer *et al*., 2000). Among protozoa, *Balantidium coli*, the ciliate parasite is known to cause severe enteropathies and has been linked to dysentery-like symptoms in livestock (da Silva Barbosa *et al*., 2015). The presence of *Eimeria* spp., in both populations suggests a natural endemicity of this parasite among buffaloes. The frequent occurrence of multiple protozoa in diarrheic calves further underlines their likely contribution to gastrointestinal disease.

A noteworthy outcome of the study was the identification of co-infections in 26.72% of infected calves, with single infections being the most common (30.56%), followed by double (10.72%), triple (6.88%), and up to hexacle infections (1.76%). Co-infections are known to exacerbate clinical symptoms, hinder immune response, and complicate treatment protocols (Ezenwa and Jolles, 2015).

The detection of multiple parasite species in diarrheic calves highlights the polyparasitic nature of infections in endemic areas and underlines the need for integrated control measures. A considerable proportion of calves experienced polyparasitism, with double, triple, and even hexacle infections recorded. Co-infection patterns differed by region double infections were most common in coastal areas, whereas quadruple and higher-order infections were more common in free-ranging populations. Polyparasitism can alter host-pathogen interactions, immune responses, and infection outcomes. Some interactions may be synergistic, while others may be antagonistic (Jolles *et al*., 2008; Ezenwa and Jolles, 2015). For example, co-infection with helminths and *Mycobacterium bovis* in African buffaloes has been shown to increase mortality (Jolles *et al*., 2008). The specific interactions between protozoa and helminths in the present study, however, remain speculative due to the lack of pathological data.

Higher parasite diversity and prevalence in free-ranging buffaloes may be due to increased exposure to contaminated water, pastures, and interactions with other livestock or wildlife. Free movement facilitates the spread of parasitic eggs and larvae via defecation in open fields and water bodies. Trematodes such as *Fasciola* and *Paramphistomum* spp. are known to be transmitted via ingestion of metacercariae from wetland vegetation or contaminated water sources (Mahato *et al*., 2005). *Eimeria* spp. transmission is also linked to exposure to stagnant or slow-moving water contaminated with oocysts.

Conversely, semi-captive systems, despite restricted movement, also showed surprisingly high infection rates. This may be due to poor indoor management, overstocking, lack of regular deworming, and unhygienic housing conditions. Most sheds surveyed were poorly ventilated, built with wood or mud, and retained moisture, all of which promote parasite development and reinfection (Kumar *et al*., 2013). Additionally, the close co-housing of adult and young animals increases the potential for transmission of parasitic infections.

Table 5: Sex wise prevalence of GI parasites of buffalo diarrheal calves.

Parameters	Name of the parasites	No. of animal infected/ Examined	Prevalence (%) (95% CI Range)	EPG Mean± SE	Odds ratio (OR)	p value
Male (356)	Amphistomes	52/356	14.61 (4.12-9.36)	21.11 ± 7.51	Male VS Female 1.029 (0.71-1.34)	<0.001
	Toxocara vitulorum	28/356	7.87 (3.67-8.69)	12.17 ± 6.69		
	Strongyloides sp.	36/356	10.11(1.90-5.96)	17.1 ± 12.6		
	Capillaria sp.	25/356	7.02(2.12-6.31)	6.00 ± 3.79		
	Trichuris sp.	49/356	13.76(2.12-6.31)	2.902 ± 0.976		
	Fasciola sp.	23/356	6.46(0.52-3.42)	8.63 ± 3.14		
	Moniezia sp.	33/356	9.27(4.59-10.02)	39.5 ± 28.3		
	Eimeria sp.	102/356	28.65(5.76-11.65)	33.48 ± 9.67		
	Giardia sp	36/356	10.11(0.89-4.17)	67.5 ± 56.3		
	Cryptosporidium sp.	29/356	8.15(2.99-7.68)	17.13 ± 8.51		
	Balantidium coli	62/356	17.42(4.12-9.36)	17.49 ± 6.89		
	Sub Total	203/356	57.02			
Female (n=269)	Amphistomes	36/269	13.38(5.19-11.91)	9.64 ± 2.25		<0.001
	Toxocara vitulorum	14/269	5.20(3.67-8.59)	7.33 ± 2.41		
	Strongyloides sp.	20/269	7.43(1.90-5.96)	10.03 ± 4.17		
	Capillaria sp.	7/269	2.60(2.12-6.31	18.77 ± 7.59		
	Trichuris sp.	18/269	6.69(2.12-6.31)	4.40 ± 1.35		
	Fasciola sp.	9/269	3.35(.52-3.42)	5.39 ± 2.44		
	Moniezia sp.	21/269	7.82(4.59-10.02)	44.8 ± 28.4		
	Eimeria sp.	53/269	19.70(5.76-11.65)	10.50 ± 2.12		
	Giardia sp	15/269	5.58(.89-4.17)	4.36 ± 1.87		
	Cryptosporidium sp.	15/269	5.58 (2.99-7.68)	11.67 ± 3.88		
	Balantidium coli	41/269	15.24(4.12-9.36)	17.10 ± 6.41		
	Subtotal	149/269	55.39			

Regional variation in prevalence was statistically significant, with Bhola district exhibiting the highest rate (65.8%), followed by Jamalpur (43.51%) and Rangpur (42.73%). These differences may reflect variations in ecological conditions, grazing practices, sanitation, and veterinary care availability. Similar findings have been reported by Biswas *et al.* (2021), where coastal and low-lying floodplain areas in Bangladesh demonstrated higher parasitic burdens due to favorable conditions for intermediate hosts and parasite survival.

Age-wise prevalence analysis revealed a higher infection rate in younger calves (1–3 months; 69.2%) compared to older calves (3–6 months; 34.27%), indicating a strong age-associated susceptibility. Younger calves may have underdeveloped immune systems and higher exposure to infective stages through milk, bedding, and communal housing, as supported by previous studies (Wadhwa *et al*., 2011; Shrestha *et al*., 2020). The higher EPG values in younger calves further emphasize the greater infection intensity in this group, warranting early prophylactic intervention and management.

In the current study, younger calves (0–3 months) exhibited significantly higher infection rates of both protozoa and helminths, corroborating findings from Sri Lanka and Australia. This may be attributed to immature immune systems and increased exposure following weaning. Conversely, findings from Nepal and Pakistan reported higher prevalence in older calves (Habib *et al*., 2017). The management practices reported by local farmers, such as allowing calves to graze only after weaning and infrequent deworming, likely contribute to the elevated parasite burden in young animals. Enhanced acquired immunity with age may reduce susceptibility in adult buffaloes (Mamun *et al*., 2011).

In contrast, sex-wise analysis showed no significant difference in overall infection rates between males (57.02%) and females (55.39%) (OR = 1.029, $p > 0.05$). However, males exhibited slightly higher prevalence and EPGs for most parasites, which could be due to hormonal or behavioral differences affecting immunity and exposure (Becker *et al*., 2004). Sex, however, did not appear to significantly influence infection rates, as supported by several previous studies (Habib *et al*., 2017; Singh *et al*., 2006). These findings are in line with observations in cattle and buffaloes in other South Asian regions (Thapa *et al*., 2022), suggesting that sex alone may not be a strong predictor of GI parasitic infection, though it may influence infection dynamics. This study conducted post-monsoon, a season favorable for the development and transmission of GI parasites due to high humidity and temperature that support the survival of infective stages in the environment (Nath *et al*., 2016).

Furthermore, feeding practices involving rice straw proven to carry infective *Fasciola* and *Paramphistomum* metacercariae that may also contribute to infections (Mahato *et al*., 2005). Shared water sources and grazing lands between buffaloes and other domestic or wild herbivores could facilitate interspecific transmission, as similar parasites (e.g., *Strongyloides* spp., strongyles) have been reported in goats and sheep in the same regions.

Taken together, these findings emphasize the widespread occurrence and diversity of GI parasites in diarrheic buffalo calves in Bangladesh, with significant variation by age and region but not by sex. The high prevalence of both helminths and protozoa, along with a substantial rate of mixed infections, underscores the urgent need for region-specific, age-targeted, and seasonally timed deworming protocols, improved husbandry practices, and awareness among farmers to mitigate economic losses and improve calf health.

CONCLUSION

This study underscores a high prevalence and wide diversity of GI parasites in diarrheic buffalo calves in Bangladesh, with marked regional and age-wise variations. The results point to critical management and environmental factors contributing to infection, including poor housing, inadequate deworming, and risky feeding practices. Given the economic importance of buffaloes in Bangladesh and the increasing intensity of farming, these findings call for immediate attention to integrated parasite control strategies tailored to local farming systems.

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NOVELTY STATEMENT

This study provides the first comprehensive assessment of the prevalence and diversity of gastrointestinal parasites specifically associated with diarrhea in buffalo calves reared under extensive buffalo farming systems in Bangladesh. Unlike previous studies that examined general parasitic infections, this research uniquely links parasite occurrence with diarrheic conditions in calves, offering a disease-focused epidemiological perspective. The study integrates field-based observations, laboratory confirmation, and system-specific risk factor analysis, generating new evidence on parasite diversity, dominance patterns, and infection dynamics within extensive management settings. By highlighting parasites that are most strongly associated with diarrheal outbreaks, the findings fill a critical knowledge gap and contribute novel insights needed for targeted parasite control, improved calf health, and sustainable buffalo farming in Bangladesh.

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: 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.

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Data availability
Data will be made available to readers upon request from the corresponding author.

Ethical statement
The study protocol was approved by the Animal Welfare and Experimental Ethical Committee (AWEEC) of Bangladesh Agricultural University (Approval No. AWEEC/BAU/2022-01). All procedures were carried out in accordance with the committee's guidelines.

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/2022-01). All procedures were carried out in accordance with the committee's guidelines.

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. This paper utilised AI-assisted technologies strictly as supportive tools to enhance clarity, structure and language quality only.

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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