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Molecular investigation of *Blastocystis* in children and calves in Bangladesh

Md Robiul Karim¹, Anas Bin Harun¹, Abdullah Al Bayazid¹, S. H. M. Faruk Siddiki¹, Junqiang Li^{2*} and Longxian Zhang^{2*}

Abstract

Background *Blastocystis*, a widely distributed zoonotic protozoan infecting both humans and numerous animals, remains poorly understood with its potential medical and veterinary significance. This study examined the molecular occurrence and genetic variation of *Blastocystis* in children and calves in Bangladesh to explore cross-species transmission and disease burden.

Methods In total, 998 DNA samples were investigated, comprising 299 stool DNA from children and 699 fecal DNA from calves, using polymerase chain reaction and sequencing of the small subunit ribosomal RNA (SSU rRNA) gene.

Results This study detected *Blastocystis* in 5.35% of the children and 14.74% of the calves. While slight variations in occurrence rates were observed across different study variables, none were statistically significant. The occurrence was highest among children under 5 years and calves aged 1–3 months. Regarding breed, the Holstein Friesian cross and the Jersey cross exhibited higher rates of infection. Conversely, occurrences were lower among children and calves in Gazipur district. The remaining parameters displayed nearly equivalent percentages of *Blastocystis*. The subtypes identified in children included ST1, ST3, and ST4, with ST1 comprising 50% of them. ST3 and ST4 were also found in calves, alongside ST10 (55.34%) being the most prevalent. Other subtypes found in calves were ST14, ST21, and ST24–ST26.

Conclusions This study found that *Blastocystis* is more common in calves than in children in Bangladesh, with genetic diversity of nine subtypes. The common occurrence of identical variants of two subtypes in both populations suggests potential zoonotic transmission, highlighting the necessity for further molecular investigations and comprehensive measures within the One Health framework to mitigate public health risks.

Keywords *Blastocystis*, Occurrence, Subtype diversity, Zoonotic potential, Children, Calves, Bangladesh

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Background

Blastocystis, an anaerobic single-celled organism, is an enteric protist parasite ubiquitous in both humans and various animals [1, 2]. Despite numerous efforts to classify *Blastocystis*, its taxonomy and pathogenicity remain elusive, demonstrating the genetic diversity and intricate evolutionary history of the organism [3–5]. Forty-six different subtypes (STs) of *Blastocystis* have been reported (ST1–ST46), each with unique genetic traits and potentially varying characteristics [4, 6]. However, only 42 of these subtypes are considered legitimate with ST18–ST20 and ST22 considered invalid. Among these 42 subtypes, 14 (ST1–ST10, ST12, ST14, ST16, and ST23) have zoonotic implications [7, 8].

In recent decades, researchers have increasingly focused on the pathogenicity of *Blastocystis*. Symptoms such as diarrhea, bloating, abdominal pain, irritable bowel syndrome, nausea, acute urticaria, ulcerative colitis, and the development of colorectal cancer have been linked to blastocystosis. Moreover, some patients may remain asymptomatic [9, 10]. Interestingly, however, *Blastocystis* is also being linked to a healthy gut microbiome [11]. Individuals with symptoms often carry not only *Blastocystis* but also other parasites, making it unclear which organism is causing the symptoms. Consequently, there is no concrete evidence that *Blastocystis* poses a public health risk, leading to a growing consensus that it is a commensal rather than a pathogen. Nevertheless, *Blastocystis* is widespread in both human and animal hosts. It has also been detected in the environment, including soil, water, and vegetables [12, 13]. Due to its ubiquity, *Blastocystis* serves as an excellent model for studying transmission dynamics and One Health approaches.

The transmission patterns of *Blastocystis* are closely linked with its life cycle, with transmission mainly occurring through the fecal-oral route. Importantly, *Blastocystis* is infectious only in its cyst form [14], allowing it to thrive in environmental reservoirs and spread through contaminated food, water, and other sources [15, 16]. The growing interaction between humans and animals, fueled by factors such as agriculture, urbanization, industrialization, and globalization, creates opportunities for cross-species transmission with implications for the emergence and spread of zoonotic diseases, including blastocystosis [17]. Globally, *Blastocystis* infection rate has been reported to range from 0.5 to 100% in humans, with some studies showing a high prevalence above 80%, possibly due to poor hygiene practices in lower-income communities as well as zoonotic and human-to-human transmission through the fecal-oral route [18]. Meanwhile, the prevalence of *Blastocystis* in cattle was found to be around 25% worldwide [18]. The occurrence of *Blastocystis* has been increasingly associated with demographic

factors, including age, gender, and education level among humans, as well as specific breeds of cattle [19]. In addition to the sociodemographic elements, close contact with infected animals, water source quality, and direct human contact are considered significant risk factors [20].

In Bangladesh, *Blastocystis* may pose significant public health risks with up to 80% infection rate, particularly among vulnerable populations such as malnourished individuals living in overcrowded slum areas [21]. The infection itself may cause gastrointestinal disorders and eventually lead to malnutrition, creating a vicious cycle of health deterioration. While studies on *Blastocystis* in humans [21–23] and zoo animals [24] have been conducted separately in Bangladesh, there is a lack of research focusing on molecular detection among livestock and children from different parts of the country. In this context, this study identified the occurrence rate and genetic variations of *Blastocystis* in children and calves in four different locations in Bangladesh. Furthermore, we aimed to explore different variables influencing the transmission of *Blastocystis* between humans and animals, contributing to targeted interventions for reducing the occurrence of *Blastocystis* and zoonotic transmission.

Methods

Sample and PCR amplification

We examined 998 fecal DNA samples, with 299 from children and 699 from calves, as part of our earlier research on *Giardia* [25], *Enterocytozoon* [26], and *Cryptosporidium* [27]. The minimum sample sizes for this study were calculated using the formula $n = Z^2 P(1 - P) / d^2$, with prevalences of 10% for children and 30% for calves, aiming for a precision of 5% at a 95% confidence level. The required minimum sample sizes were calculated to be 139 for children and 323 for calves. The fecal samples were collected randomly from children and calves in the districts of Gazipur, Sirajganj, Pabna, and Dhaka (Central Cattle Breeding and Dairy Farm) (Fig. 1). The study was designed in such a way that children were from the same household from where samples of calves were collected. Consequently, most of the children's samples were obtained from the same household.

Blastocystis was identified through polymerase chain reaction (PCR) of the small subunit ribosomal RNA (SSU rRNA) gene using the TaKaRa Taq DNA Polymerase (TaKaRa, Japan) enzyme. The 25 μ L of total reaction volume comprised 2.5 μ L of 10x PCR Buffer, 2 μ L of dNTP mixture, 0.2 μ L of enzyme, 0.4 μ L of forward (RD5: 5'-A TCTGGTTGATCCTGCCAGT-3') and reverse (BhRDr: 5'-GAGCTTTTAACTGCAACAACG-3') primers, 2 μ L of template DNA, and 17.5 μ L of PCR-grade water [28]. PCR amplification was performed at an annealing temperature of 55 °C for 45 s per cycle. The accuracy of

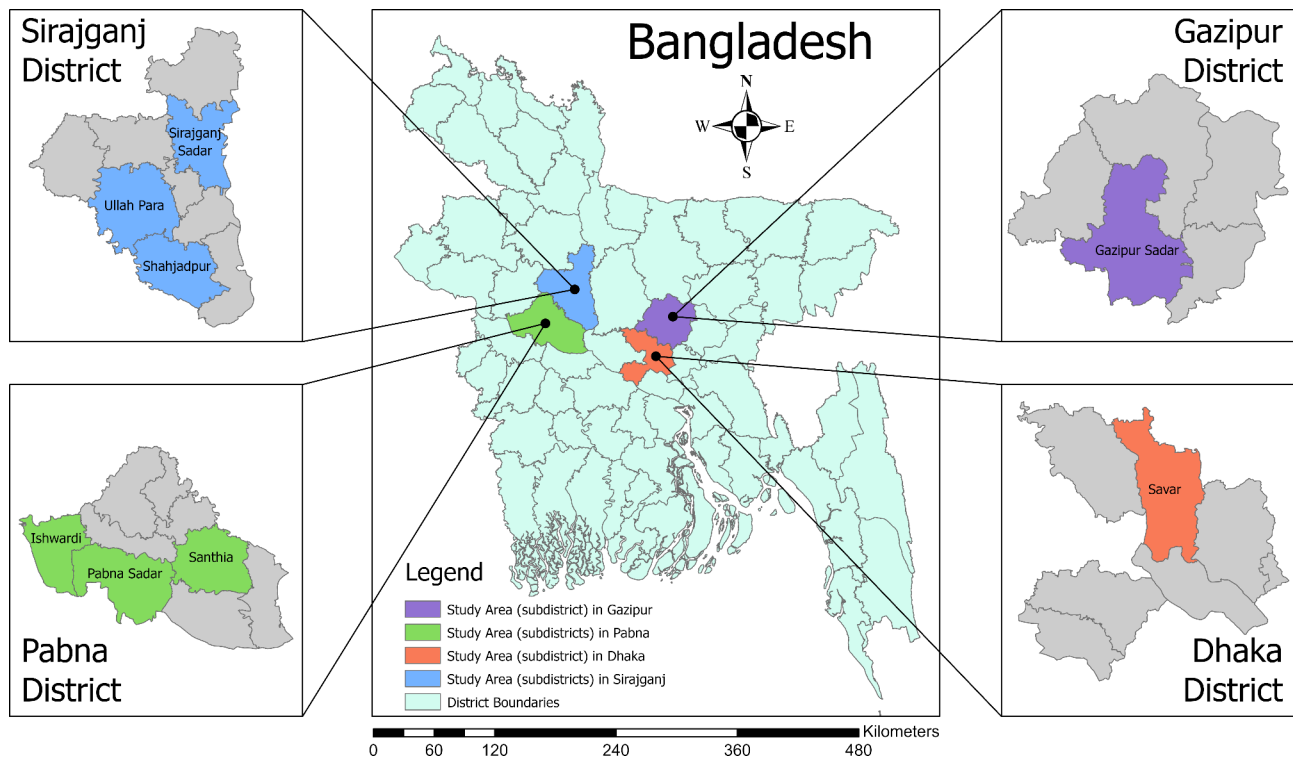


Fig. 1 Locations of the study area in Bangladesh

the results was validated through three repeated tests, with positive control (DNA from calf feces) and negative control (distilled water) incorporated into each PCR batch.

DNA sequencing and phylogenetic analysis

The PCR products with the desired band size were delivered to Sangon Biotech Co., Ltd. (Shanghai, China) for bidirectional sequencing. The resultant forward and reverse sequences were viewed and edited using ClustalW and ChromasPro software (Technelysium Pty Ltd., South Brisbane, Australia). The consensus sequences were queried against the GenBank database using the Basic Local Alignment Search Tool (BLAST) to identify matches. The subtypes of *Blastocystis* isolates were determined using the *Blastocystis* locus/sequence definition database in the PubMLST [29].

The phylogenetic analysis was conducted with 29 characteristic sequences from this study and 43 reference sequences from the GenBank. Following alignment and analysis, the sequences were processed using MUSCLE, embedded in the MEGA11 program [30]. Following alignment trimming with trimAl, which was facilitated by the online platform Phylemon 2.0 [31], the *Blastocystis* isolates of this study were subjected to phylogenetic analysis using neighbor-joining (NJ) methodology to contrast with known subtypes. The robustness of clusters was assessed using a bootstrap method with 1,000 replicates.

The tree was outrooted with *Karotomorpha* (DQ431242). Thus, the *Blastocystis* subtypes were further confirmed by phylogenetic analysis.

Statistical analysis

The Chi-squared test and multivariate logistic regression analyses were performed to investigate the possible association between *Blastocystis* occurrence and variables such as age, gender, and location of children and calves, as well as breed, feces conformation, and health status of the calves. A $p < 0.05$ value was set as the significant threshold and the analysis was carried out using the stats and associated packages in the R programming language.

Results

Occurrence of *Blastocystis* in children and calves

Out of 299 stool samples collected from children, 16 tested positive for *Blastocystis*, resulting in an occurrence rate of 5.35% (Table 1). The association of study regions, genders, and age groups with *Blastocystis* occurrence was not statistically significant. Meanwhile, *Blastocystis* was found in 14.74% (103/699) of the calves. The prevalence rates did not exhibit significant variations based on study location, gender, age, breed, fecal consistency, or health status (Table 2). No significant associations of various factors with the occurrences of *Blastocystis* were found in the multivariate logistic regression analyses either (Tables 3 and 4).

Table 1 Occurrence and subtype distribution of *Blastocystis* in children. Association of several variables with *Blastocystis* occurrence in children has been explored using chi-squared test

Variables	No. of samples	Positives (%)	Chi-square	p	Subtypes (n/GenBank accession numbers)		
					ST1	ST3	ST4
Location							
Sirajganj	140	8 (5.7)	0.330	0.848	5 (PP581301–PP581303)	2 (PP581304, PP581305)	1 (PP581308)
Pabna	85	5 (5.9)			2 (PP581301, PP581302)	2 (PP581304)	1 (PP581308)
Gazipur	74	3 (4.1)			1 (PP581303)	1 (PP581305)	1 (PP581308)
Gender							
Male	121	7 (5.8)	0.076	0.783	4 (PP581301–PP581303)	4 (PP581304, PP581305)	1 (PP581308)
Female	178	9 (5.1)			4 (PP581301–PP581303)	1 (PP581305)	2 (PP581308)
Age group							
< 5 years	124	9 (7.3)	1.521	0.217	4 (PP581301–PP581303)	1 (PP581304)	2 (PP581308)
5–14 years	175	7 (4)			4 (PP581301, PP581303)	4 (PP581304, PP581303)	1 (PP581308)
Total	299	16 (5.35)	-	-	8	5	3

Blastocystis subtypes in children and calves

The SSU rRNA gene was successfully sequenced in all *Blastocystis* isolates from children and calves. The sequence analysis identified three *Blastocystis* subtypes (ST1, ST3, and ST4) in children (Table 1). The predominant subtype in children was ST1 (50%). In calves, eight subtypes (ST3, ST4, ST10, ST14, ST21, ST24, ST25, and ST26) were identified, with ST10 (55.34%) being the most prevalent subtype (Table 2). Identical variants of two subtypes, ST3 and ST4, were identified in both children and calves from the same locations.

Phylogenetic analysis

The phylogenetic analysis illustrated the clustering of sequences from nine identified subtypes (ST1, ST3, ST4, ST10, ST14, ST21, ST24, ST25, and ST26) with their respective reference subtype sequences (Fig. 2). The constructed tree delineated isolates belonging to the same subtype, clustering together with substantial bootstrap support, thereby affirming the independent monophyletic status of the nine subtypes.

Discussion

Although numerous studies worldwide have reported variable prevalence rates of *Blastocystis* (Fig. 3), there are only a limited number of epidemiological studies conducted in Bangladesh. The molecular occurrence of *Blastocystis* infection among children in this study was found to be 5.45% (16/299). A microscopic study conducted in the Mirpur slum revealed a higher occurrence of *Blastocystis* (14%) among children, likely due to poor hygiene, inadequate healthcare, overall lifestyle factors, and different identification techniques [21]. The finding of our study aligned somewhat with a study from Nepal, where the formalin-ether concentration technique detected *Blastocystis* in 4% of children [32]. However, a molecular study revealed that 25.6% of children with gastrointestinal symptoms in Nepal carried *Blastocystis*

[33]. Similarly, in India, a microscopic study reported an occurrence rate of 14.7% among children [34]. The rate was substantially higher in a molecular study among adults in Dhaka with a rate of 78.6% [23], possibly linked to their habitat in the slum area. Another microscopic study in Dhaka reported a rate of 36.4%, with most participants being immunocompromised patients with HIV. In neighboring countries, India and Nepal, the molecular occurrence rates were similar, at 27% [35] and 26.1% [36], respectively. A Chinese systematic review reported a slightly lower occurrence rate of 3.37% [37]. In contrast, a study encompassing children from six different countries (Azerbaijan, Jordan, Nigeria, Sudan, and Tanzania) found a much higher overall prevalence of 36% [38]. Children from Brazil exhibited a notably high pooled prevalence of 86.63%, possibly influenced by the timing of the study following a gastroenteritis outbreak in the region [39]. In northern Thailand, prevalence rates varied significantly, ranging from 67 to 89% across different time points, likely reflecting the impact of environmental factors in rural areas. Meanwhile, children in Panama and China showed occurrences of 21.2% [40] and 14.3% [41].

Regarding calf fecal samples, 103 out of 699 samples tested positive for *Blastocystis*, indicating a prevalence rate of 14.74%. To the best of our knowledge, this is the first molecular study on *Blastocystis* in calves in Bangladesh. The outcome of the study was almost similar to the occurrence in Nepal (16.67%) [16]. However, the rate was lower in molecular studies conducted in Korea (6.7%) [42], Iran (9.6%) [43], and China (10.3%) [44]. In Colombia, two studies on *Blastocystis* reported occurrences of 80% and 77.58% [45, 46]. Similarly, the rates were higher in Lebanon (63.4%) [47], Japan (54.1%) [48], and Turkey (58.7%) [49]. Occurrences below 50% were reported in nations such as Brazil (21.4%) [50], the United Arab Emirates (22.7%) [51], Spain (32.1%) [52], Italy (33%) [53], and Malaysia (34.5% and 43.8%) [10, 54]. Interestingly, the infection rate was even higher in Indonesia, where

Table 2 Occurrence and subtype of *Blastocystis* in calves. Association of several variables with *Blastocystis* occurrence in calves has been explored using chi-squared test

Variables	No. of samples	Positives (%)	Chi-square	p	Subtypes (n/GenBank accession numbers)	ST3	ST4	ST10	ST14	ST21	ST24	ST25	ST26
Location													
Sirajganj	213	34 (16)	3.977	0.264	3	(PP581306)	2	17 (PP581309, PP581310, PP581312, PP581314, PP581318)	2	2	1 (PP581324)	1	6 (PP581320, PP581325, PP581329)
Pabna	344	54 (15.7)		1	(PP581306)	1	28 (PP581309–PP581313, PP581316, PP581317, PP581319)	0	0	3	12 (PP581321, PP581324, PP581326, PP581327)	2	7 (PP581320, PP581329)
Gazipur	52	3 (5.8)		0	1	(PP581309)	1	1 (PP581309)	0	0	0	0	1 (PP581320)
CCBDP ^a (Dhaka)	90	12 (13.3)		0	1	(PP581307)	11	11 (PP581309–PP581311, PP581315)	0	0	0	0	0
Sex													
Male	352	48 (13.6)	0.682	0.409	0	3	31 (PP581309–PP581316)	1	2	2	7 (PP581321, PP581324, PP581326, PP581327)	2	4 (PP581320, PP581329)
Female	347	55 (15.9)		4	(PP581306)	2	26 (PP581309–PP581315, PP581317–PP581319)	1	3	3	6 (PP581321, PP581324, PP581326, PP581327)	1	10 (PP581320, PP581325, PP581329)
Age group													
< 1 month	66	7 (10.6)	2.919	0.232	1	(PP581306)	0	3 (PP581309, PP581317)	0	0	1 (PP581321)	0	2 (PP581320)
1–3 months	458	75 (16.4)		1	(PP581306)	3	44 (PP581309–PP581316, PP581319)	2	2	2	10 (PP581321, PP581324, PP581326, PP581327)	3	10 (PP581320, PP581329)
> 3 months	175	21 (12)		2	(PP581306)	2	10 (PP581309–PP581311, PP581313, PP581318)	0	3	3	2 (PP581324)	0	2 (PP581329)
Breed													
Local	153	21 (13.7)	0.385	0.943	0	2	6 (PP581309, PP581311, PP581307)	1	2	2	6 (PP581321, PP581324, PP581327)	1	5 (PP581320, PP581329)
Holstein Friesian cross	350	53 (15.1)		4	(PP581306)	1	32 (PP581309–PP581316, PP581307)	0	3	3	4 (PP581321, PP581324, PP581326, PP581327)	1	6 (PP581320, PP581325, PP581329)
Jersey cross	178	27 (15.2)		0	1	(PP581307)	18	18 (PP581309–PP581314, PP581317, PP581319)	1	0	3 (PP581321, PP581324)	1	3 (PP581320, PP581325, PP581329)
Brahman cross	18	2 (11.1)		0	1	(PP581307)	1	1 (PP581315)	0	0	0	0	0
Fecal conformation													
Formed	318	65 (20.4)	3.852	0.278	3	(PP581306)	5	34 (PP581309–PP581316, PP581318, PP581319)	2	3	7 (PP581321, PP581324, PP581326, PP581327)	3	8 (PP581320, PP581325)
Loose	126	16 (12.7)		0	0	8	8 (PP581309–PP581312, PP581317)	0	1	1	4 (PP581321, PP581324, PP581327)	0	3 (PP581320, PP581329)

Table 2 (continued)

Variables	No. of samples	Positives (%)	Chi-square	p	Subtypes (n/GenBank accession numbers)	ST3	ST4	ST10	ST14	ST21	ST24	ST25	ST26
Soft	179	20 (11.2)			13 (PP581309, PP581311, PP581312, PP581314, PP581315)	1 (PP581306)	0	13 (PP581309, PP581311, PP581312, PP581314, PP581315)	0	1 (PP581323)	2 (PP581324, PP581326)	0	3 (PP581320, PP581329)
Watery	11	2 (18.2)			2 (PP581309, PP581310)	0	0	2 (PP581309, PP581310)	0	0	0	0	0
Health status													
Healthy	611	91 (14.9)	0.097	0.756	51 (PP581309–PP581319)	4 (PP581306)	5 (PP581307)	51 (PP581309–PP581319)	2 (PP581328)	4 (PP581323)	11 (PP581321, PP581324, PP581326, PP581327)	3 (PP581322)	11 (PP581320, PP581325)
Diseased	88	12 (13.6)			6 (PP581309, PP581310)	0	0	6 (PP581309, PP581310)	0	1 (PP581323)	2 (PP581324, PP581327)	0	3 (PP581325)
Total	699	103 (14.74)	-	-	57	4	5	57	2	5	13	3	14

^aCCBDF = Central Cattle Breeding and Dairy Farm

Blastocystis showed a 100% occurrence according to the study. This raises significant questions about whether *Blastocystis* should be classified as a pathogen or a commensal protozoan [55].

Blastocystis isolates from children for this study were subtyped as ST1, ST2, and ST3, among which the most prevalent subtype was ST1 (50%), followed by ST3 (31.25%) and ST4 (18.75%). Similarly, in children from Panama, the most prevalent subtype was ST1 (42.2%), followed by ST3 (31.8%) [40]. However, Nepalese studies reported ST4 to be predominant in children, which was later changed to ST6 following consensus terminology [33, 36]. In Thailand [56], China [41], and some other Asian and African countries [38], the predominant subtype was ST3, followed by ST1 and ST2.

The identification of 16 distinct subtypes in cattle worldwide, including ST1–ST7, ST10, ST12, ST14, ST17, ST21, and ST23–ST26, emphasizes that cattle serve as a favorable reservoir for various *Blastocystis* genetic variants, including some zoonotic subtypes [18]. This study identified subtypes ST3, ST4, ST10, ST14, ST21, and ST24–ST26 in calves. Consistent with Shams et al. (2021), ST10 emerged as the prevalent subtype in this study, detected in 57 out of 103 isolates from calves (55.34%). Similarly, in China and Japan, ST10 was the prevalent subtype in cattle, followed by subtypes ST14 and ST4 [48, 57]. However, a recent study has proposed dividing some ST10 sequences as new subtypes ST42–ST44 [58].

Subtypes ST3 (PP581304, PP581306) and ST4 (PP581307, PP581308) isolated from both children and calves were found to be identical variants, suggesting a potential zoonotic transmission between the hosts from the same locations (Pabna and Sirajganj districts). However, ST10 and ST14 are considered animal-specific subtypes with no proven zoonotic significance [59]. A recent report detected ST10 and ST14 in stool samples from healthy school-going children in Senegal, but conclusive evidence regarding their zoonotic nature is still lacking [60].

For risk analysis, several factors were considered, including the location, gender, and age of children, and the location, sex, age, breed, fecal conformation, and health status of cattle. The occurrence rate did not vary significantly by any of these factors, all falling within the range of 4–6%, except for infants under 5 years of age, which exhibited a 7.3% rate of *Blastocystis*. This might indicate that younger children may be more susceptible to infection due to factors such as a less developed immune system and potentially poorer hygiene practices, as they are more likely to engage in behaviors that increase exposure to pathogens. However, these factors did not show any significant association with *Blastocystis* infection. Geographical and environmental factors were

Table 3 Multivariate logistic regression analysis of the factors associated with *Blastocystis* occurrence in children

Variables	Odds ratio	95% CI		P-value
		Lower bound	Upper bound	
Location				
Gazipur	0.756	0.191	2.988	0.690
Pabna	1.083	0.338	3.470	0.893
Sirajganj	Ref			
Gender				
Female	1.255	0.447	3.518	0.666
Male	Ref			
Age group				
< 5 years	1.894	0.674	5.320	0.226
5–14 years	Ref			

Table 4 Multivariate logistic regression analysis of the factors associated with *Blastocystis* occurrence in calves

Variables	Odds ratio	95% CI		P-value
		Lower bound	Upper bound	
Location				
Gazipur	0.362	0.106	1.243	0.106
Pabna	1.087	0.663	1.782	0.740
Savar	0.779	0.359	1.691	0.528
Sirajganj	Ref			
Sex				
Female	1.192	0.778	1.825	0.419
Male	Ref			
Age group				
< 1 month	0.904	0.353	2.316	0.834
1–3 months	1.321	0.763	2.288	0.320
> 3 months	Ref			
Breed				
BrC	1.016	0.206	5.021	0.984
HFC	1.264	0.719	2.220	0.415
JC	1.153	0.615	2.165	0.657
Local	Ref			
Fecal conformation				
Formed	0.794	0.131	4.823	0.802
Loose	0.582	0.097	3.496	0.554
Soft	0.523	0.084	3.264	0.488
Watery	Ref			
Health status				
Diseased	0.942	0.444	2.000	0.877
Healthy	Ref			

found to affect the occurrence of *Blastocystis* in cattle in a previous study [42]. The lack of statistically significant differences in prevalence based on gender, region, or breed in both populations through chi-square test and logistic regression analyses suggests a relatively uniform distribution of *Blastocystis* across the study area, potentially pointing to shared environmental or socio-economic factors that influence transmission.

Given that the children sampled were from households that reared cattle, the interaction between humans and animals may be a crucial factor in understanding transmission dynamics. Environmental factors, such as shared

water sources and sanitation practices, are critical to consider, as they can significantly influence the prevalence rates observed [15, 16]. Soil contamination from the fecal matter of infected cattle can create environmental reservoirs, increasing the risk for children who may play in these areas. These factors can contribute to the persistence of *Blastocystis* cysts in the environment, highlighting the importance of proper waste management and hygiene measures. Socio-economic factors, although not directly measured in this study, likely play a role, as households that rear cattle may have varying access to healthcare and sanitation facilities [17]. Further

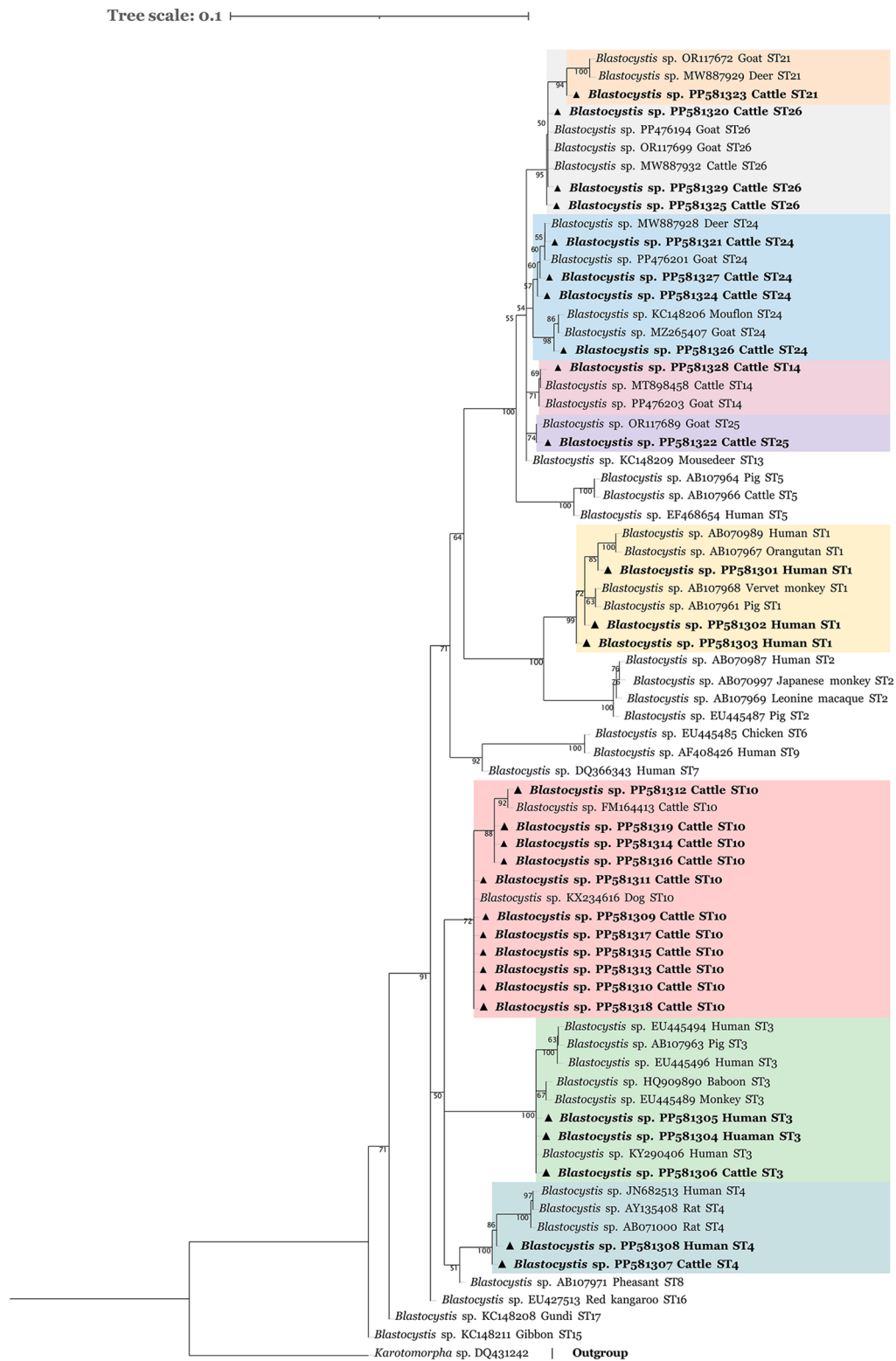


Fig. 2 *Blastocystis* isolates from children and calves and their phylogenetic relationship at the SSU rRNA gene. The subtypes found in this study are represented by filled triangles and bold texts

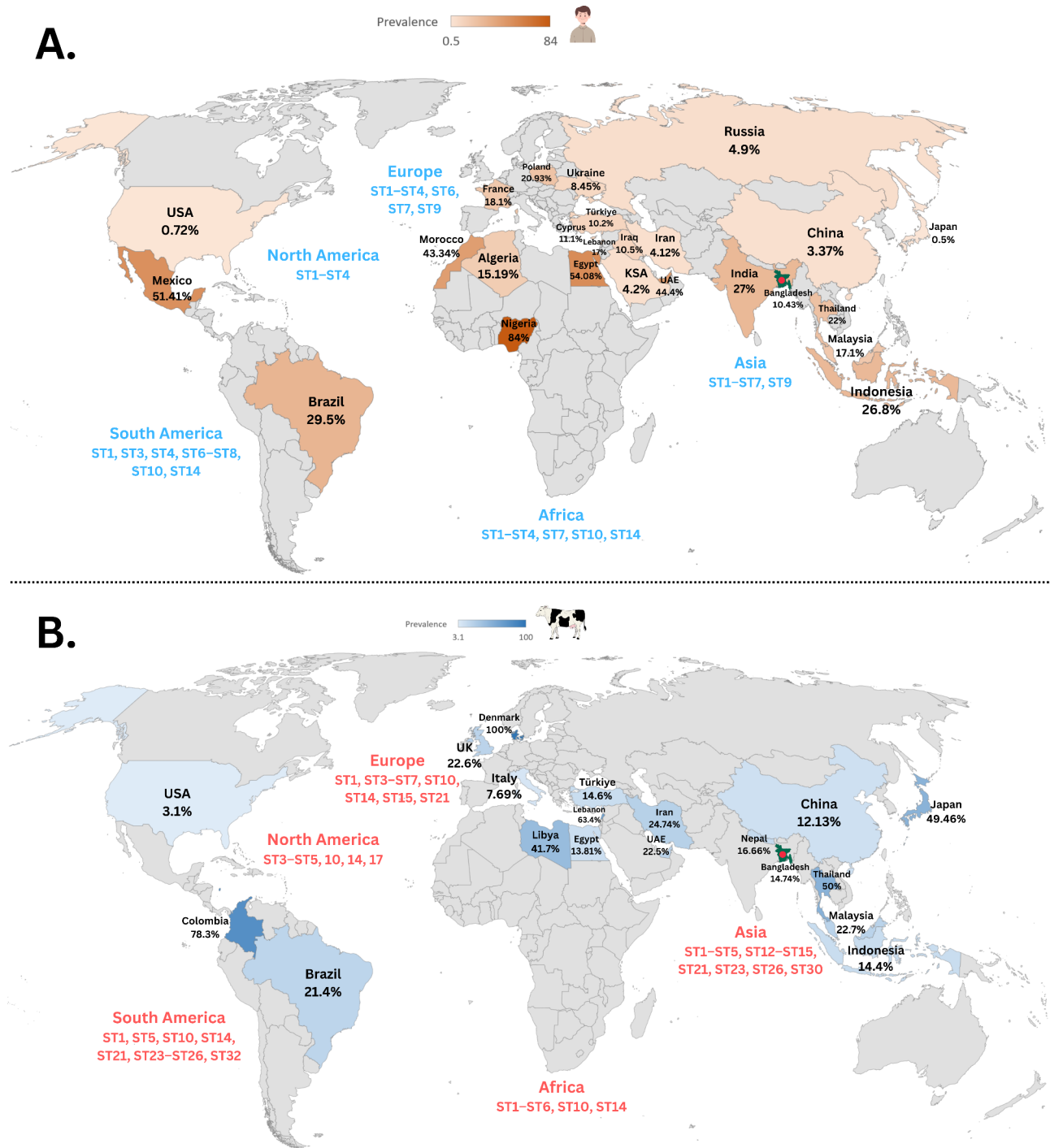


Fig. 3 Overview of global occurrence and reported subtypes of *Blastocystis* in (A) humans and (B) cattle

investigation is needed to conclusively confirm the genuine impact of these risk factors on both human and cattle populations in Bangladesh. Including environmental samples from the premises they inhabit would strengthen the study within the One Health framework.

Conclusions

Blastocystis was found to be less prevalent in children with only three subtypes (ST1, ST3, and ST4), while its occurrence was common among calves in this study. Out of the nine subtypes identified, eight (ST3, ST4, ST10, ST14, ST21, ST24, ST25, and ST26) were isolated from calves. The presence of identical variants of ST3 and ST4

in both children and calves from the same locations suggests potential zoonotic transmission through the food chain. Considering the close animal-human interaction and environmental exposure of pathogens in Bangladesh, further epidemiological studies are important for understanding the transmission pattern of this protozoan. Finally, comprehensive measures to mitigate blastocystosis should be implemented across livestock and health sectors within the One Health frameworks.

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Author contributions

MRK conceptualized, designed, acquired funding and resources, investigated, analyzed, wrote, reviewed, and edited the manuscript. ABH investigated, performed coding and illustration, analyzed, wrote, reviewed, and edited the manuscript. AAB contributed to the investigation and illustration. SHMFS was involved in sampling and reviewing the manuscript. JL conceptualized, acquired funding, wrote, reviewed, and edited the manuscript. LZ conceptualized, designed, supervised, acquired funding, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

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Data availability

All unique SSU rRNA gene sequences from this study were archived in the GenBank database of the National Center for Biotechnology Information (NCBI) under unique accession numbers. The accession numbers range from PP581301 to PP581329.

Declarations

Ethics approval and consent to participate

The Animal Research Ethics Committee (AREC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) reviewed the methodology of this study and approved it. The process of gathering data and samples started after the research objectives were explained and the owners of the calves as well as the parents or legal guardians of the children gave their written informed consent. Furthermore, this study received authorization from the Central Cattle Breeding and Dairy Farm (CCBDF) in Savar, Dhaka, and the General Hospital in the Sirajganj district.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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