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Molecular characterization and zoonotic risk assessment of *Cryptosporidium* spp. in children and calves in Bangladesh

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ABSTRACT

Cryptosporidium is a gastro-intestinal protozoan parasite that has been found to infect both humans and livestock. This study investigated the parasite in 998 fecal samples from Bangladeshi children ($n = 299$) and calves ($n = 699$) to determine its prevalence, genetic variation, and zoonotic importance. The nested PCR and sequencing of the SSU rRNA gene in the samples showed a *Cryptosporidium* infection rate of 2.3% (7/299) in children and 15.7% (110/699) in calves. Statistical analysis revealed insignificant variations in *Cryptosporidium* infections among children across age, gender, and study area, while in calves, the infection rate significantly differed based on location and breed. Genotyping of seven human isolates of *Cryptosporidium* confirmed *C. hominis* ($n = 5$) and *C. parvum* ($n = 2$). After characterizing 110 *Cryptosporidium* isolates from calves, *C. andersoni* ($n = 55$), *C. ryanae* ($n = 29$), *C. bovis* ($n = 14$), *C. parvum* ($n = 10$), *C. ubiquitum* (n = 1), and *C. occultus* (n = 1) were identified. *Cryptosporidium hominis* and *C. parvum*-positive samples were further subjected to nested PCR and sequencing of the glycoprotein 60 (*gp60*) gene for subtyping. Four *C. hominis* subtypes (IaA19R3, IaA23R3, IbA9G3, and IdA15G1) and one *C. parvum* subtype (IIdA15G1) were observed. In conclusion, *Cryptosporidium* was prevalent in calves but less common in children in the study locations, and the presence of zoonotic *Cryptosporidium* species and subtypes in calves raises concerns regarding zoonotic transmission to humans.

1. Introduction

Gastrointestinal illnesses often manifest through diarrhea, a common symptom caused by various pathogens such as bacteria, viruses, and protozoa. Among these, *Cryptosporidium* spp. stands out as the second most prevalent contributor to diarrheal diseases, responsible for child mortality under the age of five [1]. The absence of a vaccine and the limited treatment options pose significant challenges in controlling this leading cause of waterborne and foodborne outbreaks [2]. Livestock being a reservoir and source of human *Cryptosporidium* infections underscores the necessity for improved hygienic practices in animal management and adoption of the One Health approach in molecular typing to control this zoonotic parasitic disease more effectively [3].

Thus far, 45 species of *Cryptosporidium* have been identified, with over 120 genotypes previously reported; 29 of these species were found in mammalian hosts, including 19 species and four genotypes documented in humans and at least 12 species in cattle [4–6]. Among these, *C. parvum* and *C. hominis* are frequently observed in humans, while *C. andersoni*, *C. ryanae*, *C. bovis*, and *C. parvum* are prevalent in cattle [7]. On rare occasions, certain bovine *Cryptosporidium* species like *C. bovis*, *C. andersoni*, *C. ubiquitum*, and *C. xiaoi* were reported in humans [7]. *Cryptosporidium* species, especially the prevalent ones in humans and cattle, are often subtyped using the glycoprotein 60 (*gp60*) gene sequence analysis [4,8], facilitating the identification of infection origin and transmission patterns in both human and animal populations [9]. For example, the detection of *C. parvum* subtype families IIa and IId in livestock and humans suggests that newborn calves may act as a reservoir and transmit the parasite to humans. [7].

Inadequate hygiene, poor sanitation, and repeated interaction with animals contribute to zoonosis, as *Cryptosporidium* is primarily transmitted through the fecal-oral route [10,11]. In Bangladesh, smallholding cattle farmers live in close proximity to livestock, sometimes

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sharing the same house, which may facilitate the zoonotic transmission of *Cryptosporidium*. Furthermore, the practice of open defecation and dispersed domestic animal feces in rural areas may favor the interspecies transmission of this pathogen through water contamination. *Cryptosporidium* spp. have been linked to diarrhea and the most commonly identified pathogens in diarrheic patients in Bangladesh are *C. hominis* and *C. parvum* [12,13]. A recent case-control study reported a significant rate of secondary *Cryptosporidium* infection and anthroponotic transmission in young children living in urban Bangladesh [14]. Similarly, a high occurrence of this parasite was observed in cattle in this country [15–17]. Nevertheless, zoonotic transmission of *Cryptosporidium* and its genetic diversity in Bangladesh remain inadequately documented, and surprisingly, a recent study in Mymensingh District revealed no indication of cattle-to-humans transmission of *Cryptosporidium* spp. [17]. Therefore, this study aimed to explore the frequency of *Cryptosporidium* in children and calves, as well as its species, genotypes, and subtypes, in order to assess genetic variations and possible zoonotic transmission in Bangladesh.

2. Materials and methods

2.1. Samples and PCR amplification

In this study, we used 998 fecal DNA samples from 299 children and 699 calves from our previous molecular epidemiological investigations on *Giardia* [18] and *Enterocytozoon* [19]. The DNA samples were tested for *Cryptosporidium* with amplification of the SSU rRNA gene by nested PCR [20]. Subsequently, subtyping of *Cryptosporidium*-positive samples involved amplification of the *gp60* gene using nested PCR [21]. PCR was consistently carried out using KOD -Plus-high fidelity DNA polymerase (Toyobo, Japan). The total reaction volume was 25 μL, consisting of 2.5 μL of 10× KOD -Plus- Buffer, 2.5 μL of 2 mM dNTPs, 1.5 μL of 25 mM $MgSO_4$, 0.5 μL of KOD-Plus- (1.0 U/μL), 0.5 μL of each primer (0.4 mM), 1 μL of DNA template, and 16 μL of PCR grade water. The primers and reaction conditions for both targets were described elsewhere [22]. To ensure the reliability and validity of the analysis, DNA from a monkey and distilled water were used as positive and negative controls for every PCR amplification.

2.2. Nucleotide sequencing and phylogenetic analysis

All of the secondary PCR products were sequenced in both forward and reverse directions by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences were edited and analyzed using ClustalX software, along with viewing them in both directions using Chromas version 2.6.6 DNA sequencing and analysis software (Technelysium Pty Ltd., South Brisbane, Australia). The unambiguous sequences were then investigated in the GenBank database with the Basic Local Alignment Search Tool (BLAST), and *Cryptosporidium* species and subtypes were identified.

Phylogenetic and molecular evolutionary analyses were performed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0, as described by Tamura et al. [23]. The phylogeny of *Cryptosporidium* isolates based on the SSU rRNA gene was determined through both neighbor-joining (NJ) and maximum likelihood (ML) analyses. The Kimura-2-parameter model was utilized to calculate evolutionary distances. The phylogenetic tree's reliability was evaluated using a bootstrap technique with 1000 replicates.

2.3. Statistical analysis

Data were inputted into Microsoft Excel and subsequently transferred to SPSS to conduct statistical analysis. To explore any correlations between *Cryptosporidium* infections and variables like age, sex, calf breed, and study locations, the Chi-squared test was utilized. The significance threshold was set at $p < 0.05$.

3. Results

3.1. Prevalence of Cryptosporidium spp. in children and calves

Seven (2.3%) of the 299 stool samples from children had positive test results for *Cryptosporidium*. The prevalence of *Cryptosporidium* infections showed variability across study locations, age groups, and genders (Table 1). However, differences in the prevalence based on location, gender, or age group were statistically insignificant.

In calves, the occurrence of *Cryptosporidium* was 15.7% (110/699). The prevalence rate varied according to study location, age, sex, and breed groups (Table 2). Notably, the prevalence showed significant variation across the study locations and breed groups ($p < 0.01$). Calves from Central Cattle Breeding and Dairy Farm (CCBDF) exhibited a significantly higher *Cryptosporidium* infection rate (37.8%) compared to those from other locations. Furthermore, compared to other breeds, *Cryptosporidium* was significantly prevalent in Brahman Cross calves (14.3%) (Table 2).

3.2. Cryptosporidium species identified

All *Cryptosporidium* isolates from children and calves were successfully sequenced. DNA sequence analysis of seven isolates from children identified *C. hominis* ($n = 5$) and *C. parvum* ($n = 2$) (Table 3). The analysis of 110 DNA sequences from calves revealed six *Cryptosporidium* species, namely *C. andersoni*, *C. ryanae*, *C. bovis*, *C. parvum*, *C. ubiquitum*, and *C. occultus* (Table 3). Among them, *C. andersoni* was the most common species, identified in 50.0% (55/110) of the cases, followed by *C. ryanae* (26.4%, 29/110), *C. bovis* (12.7%, 14/110), *C. parvum* (9.1%, 10/110), *C. ubiquitum* (0.9%, 1/110), and *C. occultus* (0.9%, 1/110).

Cryptosporidium species were distributed among calves based on their age. *Cryptosporidium andersoni* was found in 83.3% of the calves aged over 3 months, making it the most prevalent species in this age group. The occurrence of *C. ryanae* was higher (31.7%) in the calves of 1–3 months of age. Similarly, in the age group of 1–3 months, *C. bovis* had a higher occurrence (13.4%) (Table 3).

3.3. Sequence analysis of Cryptosporidium spp

The analysis of the SSU rRNA gene sequences unveiled the existence of intra-species variations within some *Cryptosporidium* species. There was no intra-species sequence variation in five *C. hominis* isolates and 12*C. parvum* isolates, each representing a single sequence type and sharing 100% identity with a wastewater isolate from China (KJ019854) and a human isolate from India (MW116650), respectively.

Three distinct variants of the SSU rRNA gene, designated as Type I ($n = 28$), Type II ($n = 22$), and Type III ($n = 4$), were detected in 55*C. andersoni* isolates. *Cryptosporidium andersoni* Type I (MK982464) had a 100% similarity with the sika deer isolate from China (KX259130). Type II (MK982465) showed 100% similarity to the yak isolate from

Table 1
Prevalence of *Cryptosporidium* infection in children.

Variables	Total sample	Positive (%)	χ^2	*p* value
Study area				
Sirajganj	140	4 (2.9)	0.480	0.787
Pabna	85	2 (2.4)		
Gazipur	74	1 (1.4)		
Gender				
Male	178	3 (1.7)	0.270	0.603
Female	121	4 (3.3)		
Age group				
< 5 years	175	5 (2.9)	0.098	0.754
5–14 years	124	2 (1.6)		
Total	**299**	**7 (2.3)**		

Note: χ^2 and *p* values were calculated to assess the prevalence variations in children across different study areas, genders, and age groups.

Table 2
Prevalence of *Cryptosporidium* infection in calves.

Variables	Total sample	Positive (%)	χ^2	*p* value
Study area				
Sirajganj	213	34 (16.0)	41.898	0.000**
Pabna	344	39 (11.3)		
Gazipur	52	3 (5.8)		
CCBDF (Savar, Dhaka)	90	34 (37.8)		
Sex				
Male	352	45 (12.8)	4.224	0.040
Female	347	65 (18.7)		
Age group				
<1 month	66	10 (15.2)		
1–3 months	458	82 (17.9)	5.561	0.62
>3 months	175	18 (10.3)		
Breed group				
Local	153	9 (3.1)		
HFC	350	63(13.5)	15.551	0.001**
JC	178	36 (5.6)		
BrC	18	2 (14.3)		
Total	**699**	**110 (15.7)**		

Note: χ^2 and *p* values were calculated to assess the prevalence variations in calves across study areas, sexes, ages, and breeds; HFC, JC, and BrC represent Holstein Friesian Cross, Jersey Cross, Brahman Cross breeds, respectively; ** represents statistically significant difference ($p < 0.01$); CCBDF represents Central Cattle Breeding and Dairy Farm.

China (MK139948). However, Type III (MW043437) was a new sequence with one nucleotide substation at position 658 (A-to-G) differing from the human isolate (KF271469) from China.

The sequences of 29*C. ryanae* isolates were classified into three sequence types: Type I ($n = 15$), Type II ($n = 13$), and Type III (n = 1). Specifically, Type I (MK982468) exhibited identity with a calf isolate from China (HQ179574), while Type II (MW043439) and Type III (MW043440) shared identical sequences with isolates from calves in Ethiopia (KT922233) and China (KP793013), respectively.

The analysis of 14*C. bovis* sequences revealed two sequence types, such as Type I ($n = 2$) (MK982466) and Type II ($n = 10$) (MW043438), which were 100% similar to the sequences of calves from China (JX515546) and Ethiopia (KT922231), respectively. Similarly, the sequences of *C. ubiquitum* (MW043441) and *C. occultus* (MK982467) were found to match the reference sequences identified from lamb in Ethiopia (KT922236) and rats in the Czech Republic (MG699179), respectively.

3.4. *Cryptosporidium hominis and C. parvum subtypes*

Sequencing of the *gp60* gene was successful in all five *C. hominis* isolates from children and six (two from children and four from calves) of the 12*C. parvum* isolates. Analysis of the *C. hominis* sequences identified four known subtypes under three subtype families, including IaA19R3, IaA23R3, IbA9G3, and IdA15G1 (Table 4). The sequences of subtypes IaA19R3 and IaA23R3 showed 100% similarity with reference *C. hominis gp60* subtypes isolated from humans in India (JF268624) and Nigeria (JQ798143), respectively. The subtype IbA9G3 was identical to a human isolate reported from Australia (JF727787). Meanwhile, the subtype IdA15G1 displayed 100% similarity with the sequence (JF495139) obtained from humans in India.

The six *C. parvum* isolates represented one subtype, IIdA15G1, that originated from both children (MK982539) and calves (MW055930) (Table 4). These isolates exhibited complete identity with each other and presented 100% resemblance with sequences from humans in India (JF268648) and the United Kingdom (GU214367) and calves (KT964798), macaques (KJ917586), sheep (MH794167), and rodents (GQ121027) in various regions of China.

3.5. *Phylogenetic analysis*

The phylogenetic tree in Fig. 1 displays the relationships among our 13 representative *Cryptosporidium* SSU rRNA gene sequences and 43 reference sequences sourced from the GenBank database, representing a

Table 3
Age-wise distribution of *Cryptosporidium* species in children and calves.

Age group	No. of samples positive	*Cryptosporidium* species/No. of samples positive (%)						
		C. hominis	*C. parvum*	*C. andersoni*	*C. bovis*	*C. ryanae*	*C. ubiquitum*	*C. occultus*
Children								
< 5 years	5	4	1					
5–14 years	2	1	1					
Subtotal	**7**	**5 (71.4)**	**2 (28.6)**					
Calves								
<1 month	10		3 (0.3)	3 (0.3)	1 (0.1)	3 (0.3)	0	0
1–3 months	82		7 (8.5)	37 (45.1)	11 (13.4)	26 (31.7)	1 (1.2)	0
>3 months	18		0	15 (83.3)	2 (11.1)	0	0	1 (5.6)
Subtotal	**110**		**10 (9.1)**	**55 (50.0)**	**14 (12.7)**	**29 (26.4)**	**1 (0.9)**	**1 (0.9)**

Table 4
Cryptosporidium hominis and *C. parvum* subtype distribution in children and calves.

Isolate	Organism	Location	Children		Calves			Subtype family	*gp60* subtype
			Gender	Age[#]	Sex	Age*	Breed		
SUH 31	*C. hominis*	Sirajganj	M	2 y	–	–	–	Id	IdA15G1
SUH82	*C. hominis*	Sirajganj	M	5 y	–	–	–	Id	IdA15G1
PSH240	*C. hominis*	Pabna	F	1 y	–	–	–	Ia	IaA19R3
PSH271	*C. hominis*	Pabna	F	1 y	–	–	–	Ib	IbA9G3
GPH309	*C. hominis*	Gazipur	M	4 y	–	–	–	Ia	IaA23R3
SH25	*C. parvum*	Sirajganj	F	4 y	–	–	–	IId	IIdA15G1
SH55	*C. parvum*	Sirajganj	F	9 y	–	–	–	IId	IIdA15G1
SD6672	*C. parvum*	CCBDF	–	–	F	2.4 m	HFC	IId	IIdA15G1
SD6755	*C. parvum*	CCBDF	–	–	F	1.2 m	JC	IId	IIdA15G1
SD6758	*C. parvum*	CCBDF	–	–	F	1.1 m	JC	IId	IIdA15G1
SD6769	*C. parvum*	CCBDF	–	–	F	0.7 m	JC	IId	IIdA15G1

Note: Hash (#) and asterisk (*) represent age in year and month, respectively; M and F represent male and female, respectively; HFC and JC represent Holstein Friesian cross and Jersey cross breeds, respectively; CCBDF represents Central Cattle Breeding and Dairy Farm located at Savar, Dhaka.

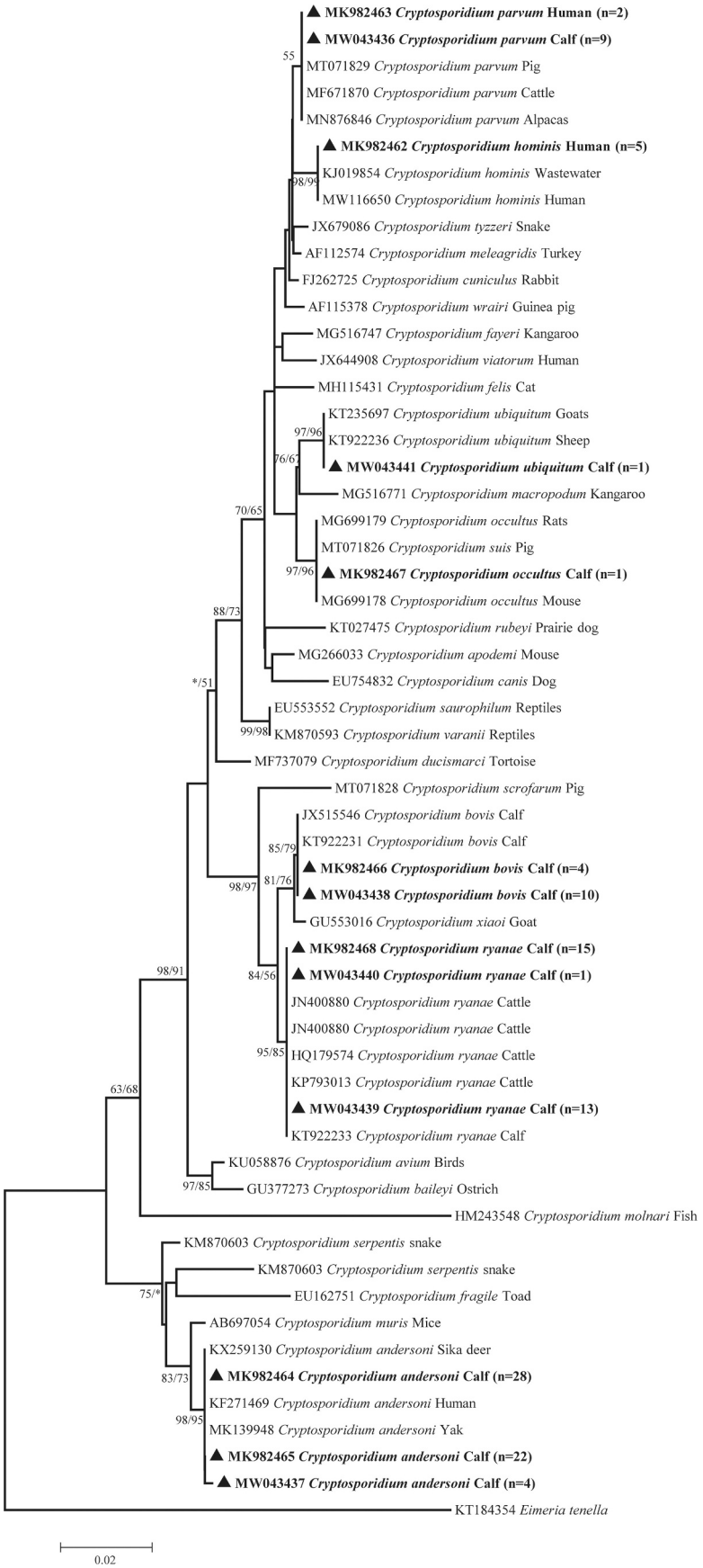

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Fig. 1. Phylogenetic relationship of the *Cryptosporidium* isolates from children and calves at the SSU rRNA gene. Both maximum likelihood (ML) and neighbor-joining (NJ) techniques were used in the phylogenetic analysis. The maximum composite likelihood technique was used to calculate evolutionary distances, which included a gamma distribution (shape parameter = 0.5) that accounted for rate variation among sites. The percentage of trees forming clusters, determined through a bootstrap test (1000 replicates), and posterior probabilities (expressed as a percentage) are specified adjacent to the branch nodes. An asterisk denotes a value below 50%; nodes with values lower than 50% in both analyses are not presented. The genotypes identified in the current study are highlighted in bold and marked with filled triangles.

spectrum of *Cryptosporidium* species such as *C. hominis*, *C. parvum*, *C. andersoni*, *C. ryanae*, *C. bovis*, *C. ubiquitum*, and *C. occultus*. To establish the tree's root, the sequence of the *Eimeria tenella* isolate (KT184354) was used. Both neighbor-joining (NJ) and maximum likelihood (ML) methods employed in the phylogenetic analysis demonstrated that the representative *Cryptosporidium* isolates from our study clustered together with their corresponding reference isolates.

4. Discussion

The occurrence of *Cryptosporidium* in children was 2.3% (7/299) in this investigation. A similar infection rate (2.4%, 8/321) was found in primary school children in Southwestern China [24]. A slightly higher infection rate of 3.5% (68/1949) was previously reported in children with diarrhea in Bangladesh [25]. Additionally, higher rates of *Cryptosporidium* infections were observed in other studies, including 5.6% (8/143) among children within the hospital system in South Africa [26], 5.2% (116/2232) in children in rural Ghana [27], and 4.8% (8/165) in children with diarrhea in Nigeria [28]. However, lower infection rates were recorded in children from Egypt (1.4%, 8/585) [29] and China (2.0%, 10/500) [30]. Regarding *Cryptosporidium* infections in calves, our study recorded a prevalence of 15.7% (110/699). Notably, higher infection rates of 83.8% (67/80) were reported in Japan among diarrheic calves [31], 55.4% (98/177) in Austrian calves [32], and 28.97% (93/321) in dairy calves in Northern China [33]. Conversely, lower infection rates of 5% (31/623) were documented in calves in Bangladesh [17], 9.7% (24/248) in dairy calves in Egypt [29], and 4.4% (14/315) in pre-weaned native calves in South Korea [34]. These variations in *Cryptosporidium* infections across different geographic regions are possibly linked to several factors like sample sizes, breeds, diagnostic methods, and farm management practices.

The analysis of human *Cryptosporidium* isolates in our study identified *C. hominis* (71.4%) and *C. parvum* (28.6%), which is consistent with the most recent studies [4,7]. *Cryptosporidium hominis* and *C. parvum* contribute to about 95% of infections among the 19 species and 4 genotypes of *Cryptosporidium* described in humans [4,35]. According to the results of our study, *C. hominis* was the predominant species among children, similar to the findings of a prior study in Bangladesh that identified 91% of cases as *C. hominis*, 7% as *C. parvum*, and 2% as *C. felis* [13]. Our findings add to the evidence that *C. hominis* is the prevalent species in developing nations. This study also revealed the presence of six species, named *C. andersoni* (50%), *C. ryanae* (26.4%), *C. bovis* (12.7%), *C. parvum* (9.1%), *C. ubiquitum* (0.9%) and *C. occultus* (0.9%) in calves. To our knowledge, our study marks the first report to identify three of these six species in cattle in Bangladesh, which are among the 12 *Cryptosporidium* species globally reported in cattle [4]. *Cryptosporidium andersoni*, *C. ryanae*, *C. bovis*, and *C. parvum* are the four most dominant species responsible for infecting cattle [6], which were also common in our study. However, a recent study in the north-central Mymensingh district of Bangladesh identified *C. andersoni*, *C. bovis*, and *C. parvum* in calves [17].

Cryptosporidium andersoni is the prevalent species in cattle [36], and it was found to be dominant in our study as well. Furthermore, consistent with the previous findings of *C. andersoni* as a common species in cattle, irrespective of their post-weaned, juvenile, and adult status [6], our study also revealed its common occurrence in the >3 month age group. Similarly, *C. ryanae* and *C. bovis* were common species infecting the 1–3 month age group, as they were frequently detected in both pre- and post-weaned calves worldwide [6]. *Cryptosporidium parvum*, on the other hand, is typically detected in pre-weaned calves [37]; however, according to our investigation, it was frequently observed in calves between one and three months old.

Cryptosporidium ubiquitum was identified in a calf sample in our study, which is prevalent in rodents, carnivores, wild and domestic ruminants, non-human primates, and humans, especially in developed countries [4]. Similarly, while *C. occultus* was identified in one of our calf samples, it seems to be widespread in bovine species, including cattle [4]. This species was also reported in a few human samples from the UK, British Columbia, and China [4]. Our findings show that all six *Cryptosporidium* species in calf samples, with the exception of *C. ryanae*, have previously been reported in humans [4], implying a potential zoonotic transmission of them from calves to humans in both organized and small-holder dairy farms in Bangladesh.

In this study, the subtyping of five *C. hominis* isolates from children revealed *gp60* subtypes IaA19R3, IaA23R3, IbA9G3, and IdA15G1, while the subtyping of six *C. parvum* isolates (two from children and four from calves) indicated subtype IIdA15G1. Previously, Hira et al. [13] identified various subtypes of *C. hominis* and *C. parvum* under subtype families Ia, Ib, Id, Ie, If, IIa, and IIm among children in Bangladesh. Among the five subtypes found in our study, IbA9G3 and IdA15G1 were previously reported in Bangladeshi children [13]. Regarding subtype distribution in calves in Bangladesh, a recent study identified *C. parvum* subtype IIdA16G1 [17], whereas we identified subtype IIdA15G1 in our calf samples. However, this subtype IIdA15G1 was previously isolated from human patients residing in the neighboring northern regions of India [38]. In other studies on *C. hominis* subtype distribution in children, similar subtypes from families Ia, Ib, and Id were often found. For example, Eibach et al. [27] found subtypes IaA21R3 and IbA13G3 in children in rural Ghana, and Naguib et al. [29] found IbA6G3, IdA17, and IdA24 in children in Egypt. However, subtype families Ie and If were also commonly reported in children in Kenya [39], Ie in children with diarrhea in Nigeria [28], subtype IeA11G3T3 in children in Mexico [40], and IfA14G1R5 in children in Egypt [29]. Meanwhile in China, diverse subtype families and numerous Ib subtypes were reported from humans [41]. Overall, in humans from developing countries, *C. hominis* subtype families Ia, Ib, Id, Ie, and If are commonly found.

While we found *C. parvum* subtype IIdA15G1 in our child samples, the distribution of the subtypes in children varied by region, including the identification of subtype family IIc in Kenya [39], subtype IIcA5G3 in rural Ghana [27], IIaA15G2R1, IIdA20G1, and IIcA5G3a in Egypt [29], IIaA15G2R1 and IIaA16G1R1 in Mexico [40], and IIa and IId in diarrheic children from Nigeria [28].

The detection of *C. parvum* subtype IIdA15G1 in calves in this study aligns with the findings of Chinese studies, which also reported the predominance of IId subtypes, particularly IIdA15G1 and IIdA19G1, in dairy calves [41]. The IId subtypes are similarly prevalent among lambs and goat kids from Europe and Middle East, as well as in dairy calves in Middle East and Sweden. In contrast, IIa subtypes are more commonly detected in cattle populations in developed countries [6].

Among the various subtypes of *C. parvum*, the primary ones with zoonotic potential belong to subtype families IIa and IId [7]. Numerous studies documented that *C. parvum* subtype IIdA15G1 caused infections in both humans and calves [38,42,43]. This particular subtype has also been widely found in various ruminant and wildlife hosts [4]. Furthermore, it is worth noting that subtype IIdA15G1 has been previously linked to cryptosporidiosis outbreak in China, resulting in calf mortality

[44]. Infection of pre-weaned calves with *C. parvum* has been associated with several instances of human cryptosporidiosis outbreaks, and there is growing evidence suggesting animal contact to be the most probable mode of transmission to humans [45–47]. The observation of subtype IIdA15G1 in both children and calves in our investigation further supports the potential zoonotic transmission between cattle and humans.

In conclusion, this study explored the molecular epidemiology and genetic variations of *Cryptosporidium* and revealed its widespread occurrence in calves with lower occurrence in children. Our study has identified six different *Cryptosporidium* species in cattle, encompassing the zoonotic *C. parvum* and less zoonotic *C. andersoni*, *C. bovis*, *C. ubiquitum*, and *C. occultus*. Notably, our research marks the first report of three of these species in Bangladesh. Detection of these species, along with the zoonotic subtype *C. parvum* IIdA15G1 in both children and calves, implies a risk for zoonotic transmission. Given the environmental exposure and close human-animal contact in Bangladesh, further molecular epidemiological investigations are crucial for elucidating transmission dynamics and associated public health implications of this protozoa.

Ethics statement

The research protocol underwent a thorough review and received approval from the Animal Research Ethics Committee (AREC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). Before proceeding with the study, objectives were clearly communicated to the guardians of the children and the owners of the calves involved. Importantly, the collection of data and samples was carried out only after obtaining written consent from the participants, ensuring their voluntary participation in the study. Furthermore, support letters from the respective authorities were obtained for sampling at the Central Cattle Breeding and Dairy Farm (CCBDF) in Savar, Dhaka, and the General Hospital in Sirajganj district.

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Authors' contributions

Conceptualization and study design: MRK and LZ; sampling: MRK, FIR, and ABH; experiment and data analysis: MRK, JL, ABH, and FIR; contributing reagents, lab supplies and analysis tools: MRK, JL, LZ, and FIR; writing and reviewing the manuscript: MRK, JL, and LZ. The manuscript was finalized after being read and approved by all authors.

CRediT authorship contribution statement

Md Robiul Karim: Conceptualization, Methodology, Funding acquisition, Investigation, Resources, Formal analysis, Writing – original draft, Writing – review & editing. **Junqiang Li:** Conceptualization, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. **Anas Bin Harun:** Investigation, Formal analysis, Writing – review & editing. **Farzana Islam Rume:** Investigation, Formal analysis, Writing – review & editing. **Longxian Zhang:** Conceptualization, Methodology, Funding acquisition, Writing – review & editing, Supervision.

Declaration of competing interest

The authors confirm that they have no conflict of interest to declare.

Data availability

All of the representative sequences from this work were deposited at the National Center for Biotechnology Information (NCBI) and are now archived in the GenBank database with specific accession numbers. Regarding the SSU rRNA gene, the accession numbers fall within the range of MK982462 to MK982468 and MW043436 to MW043441, while regarding the *gp60* gene, the accession numbers range from MK982535 to MK982539, with an additional entry, MW055930.

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