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***Research article***

# Prevalence of *Cryptosporidium* and *Giardia* species infections among children and calves in Chattogram, Bangladesh

***Zebunnahar Yasmin[1], Mohammad Alamgir Hossain[1], Sharmin Chowdhury[1], Md. Sirazul Islam[1], Nusrat Jahan Nipa [2], Md. Habib Ullah Masum[3] and AMAM Zonaed Siddiki[1,3*]***

[1]Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh;
[2]Department of Microbiology, Chattogram Medical College, Bangladesh;
[3]Nextgen Informatics Ltd, Bangladesh

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*Corresponding author:
Cell: +880 1717718884

E-mail:zsiddiki@cvasu.ac.bd

A B S T R A C T

Cryptosporidiosis and giardiasis are parasitic diseases that may significantly affect human and animal populations, notably cattle and goats. The zoonotic potential and modes of transmission of these diseases have been the subject of several epidemiological studies. We collected a total of 437 fecal specimens to determine the prevalence of cryptosporidiosis and giardiasis among human and animal populations in Bangladesh. Hence, 200 fecal samples from symptomatic children and 237 samples from calves were gathered from healthcare facilities and cattle farms in the Chattogram metropolitan area. To identify *Cryptosporidium* oocysts and *Giardia* cysts, we employed a modified Ziehl-Neelsen acid-fast staining protocol (Z-N stain) for *Cryptosporidium* and Trichrome staining for *Giardia*, followed by a polymerase chain reaction (PCR) with partial amplification of *SSU* and *tpi* gene, respectively. Based on the findings of the modified Z-N stain, the prevalence of *Cryptosporidium* infection was determined to be 13.5% among hospitalized children with diarrhea, whereas infected calves exhibited the prevalence of 23.63%. However, the *SSU* gene-based polymerase chain reaction (PCR) method revealed that the frequency of *Cryptosporidium* infection was 9.5% among hospitalized children with diarrhea and 19.41% among infected calves. Furthermore, Trichrome staining techniques indicated that the occurrence of *Giardia* in children and calves was 9.5% and 19.41%, respectively. The results obtained from *tpi* gene-based PCR analysis revealed a prevalence rate of 9% for *Giardia* infection in children and 10.55% in calves. The findings from this study can be used as baseline for other researchers to perform extensive research on cryptosporidiosis and giardiasis in both human and animal populations.

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## 1. INTRODUCTION

Cryptosporidiosis and giardiasis are parasitic diseases that may substantially impact humans and animals, particularly cattle and goats (Ryan and Zahedi, 2019). These parasites are important waterborne pathogens due to their high prevalence rate, positive correlation with waterborne outbreaks, and their resistant pattern to multiple disinfectants (Savioli et al., 2006).

The occurrence of diarrhea in Bangladesh has been associated with the existence of both *Cryptosporidium* and *Giardia* .These diseases are often caused by two distinct parasites, *Cryptosporidium* and *Giardia*, and have been attributed to sporadic and outbreak episodes of diarrhea and malnutrition (Šlapeta, 2013). However, several epidemiological studies have investigated *Cryptosporidium* and *Giardia* transmission routes and zoonotic potential (Robertson, 2009). There are different species and genotypes of *Cryptosporidium* and *Giardia* that may infect humans, while cattle are considered to be the primary source of zoonotic transmission of these parasites (Ryan et al., 2021; Kifleyohannes et al., 2022; Fayer, 2004).

Currently, there are more than 26 recognized species of *Cryptosporidium*, with more than 40 distinct genotypes that have not been classified as species yet (Ryan et al., 2014). Over 15 species of *Cryptosporidium* have been associated with human cryptosporidiosis, although *C. hominis* and *C. parvum* account for most cases worldwide. *C hominis* was shown to be the most common species responsible for diarrhea in children in research conducted in Bangladesh, Peru, Brazil, and India (Hira et al., 2011; Mbae et al., 2015; Xiao et al., 2001). A comprehensive analysis of *gp60* sequencing data regarding *Cryptosporidium*, especially in developing countries, suggested that *C. hominis* accounts for around (70-90%) of human infections (Thompson, 2008; Xiao and Fayer, 2008).

In Bangladesh, *Cryptosporidium* and *Giardia* have been linked to diarrhea (Rahman et al., 1990). In cases of giardiasis, diarrhea was primarily caused by *G. duodenalis* assemblage A (Haque et al., 2005; Perti et al., 2009). *Cryptosporidium hominis* and *C. parvum* were found in patients with diarrhea (Perti et al., 2009). All of the studies mentioned above took place in Dhaka, one of the world's fastest expanding megacities. The presence of overcrowded slums, as well as the pollution of nearby waterways with untreated wastewater (Rahman and Hossain, 2008), may enhance (waterborne) human to human transmission. *Giardia* infections in rural Bangladesh have also been linked to childhood diarrhea (Hasan et al., 2006). An earlier study discovered a significant frequency of *Cryptosporidium* and *Giardia* in cattle. Young calves are thought to be a reservoir for these parasites, and transmission of *Cryptosporidium* and *Giardia* from cattle to cattle handlers has been reported in Bangladesh and India (Khan et al., 2011).

Previous study revealed *Giardia lamblia* in 68% of toddlers aged 2 to 8 months in Bangladesh (Haque et al., 2003) and identified 11.08% *Giardia lamblia* infection in a 2-5 year old age group in Mirpur, an urban slum district in Dhaka. Several investigations in Bangladesh have been conducted to estimate the prevalence of *Giardia lamblia* by direct microscopic examination (Haque et al., 2003; Alam et al., 2011). Few studies have used current diagnostic procedures such as immunofluorescence assay, enzyme-linked immunosorbent assay (ELISA), or polymerase chain reaction (PCR). A sensitive and specific diagnostic approach is required for screening *Cryptosporidia* and *Giardia.* PCR provides higher sensitivity and specificity than traditional diagnostic procedures such as direct microscopic examination) (McGlade et al., 2003).

The current study aimed to elucidate the prevalence of cryptosporidiosis and giardiasis in both the human and animal population of Chattogram by utilizing staining and polymerase chain reaction (PCR) approaches. The study also aimed to evaluate the association of different variables with the presence of water borne cryptosporidiosis and giardiasis in human and animal populations.

## 2. MATERIALS AND METHODS

### Study area

This study was conducted on a total of 437 ($n$ = 437) fecal samples collected from both humans and cattle suffering from diarrhea. A total of 200 ($n$ = 200) human fecal samples were collected from the child patients (1 month to <5 years of age) with gastrointestinal discomfort, such as diarrhea, dehydration, abdominal pain, nausea, and vomiting, referring to the Chattogram Medical College Hospital, Chattogram. In the meantime, 237 ($n$ = 237) fecal samples were also collected from calves (1 to <6 months of age) originating from different Chattogram

metropolitan area's local cattle farms, all having diarrheagenic symptoms.

**Study group**

The specific age categories for studying *Cryptosporidium* and *Giardia* in children and calves are designed to account for developmental and behavioral differences that influence infection risk and prevalence. For children, (1-10 months) infants and very young children, (11-15 months) children begin to explore more actively**,** (16-35 months) toddlers, (> 36 months) older children. The age categories for calves (1 to 15 days**)** newborn and very young calves, (16 to 30 days) as calves grow their immune system starts to develop, (31 to 60 days) at this stage calves are transitioning to a more solid diet, (>60 days**)** older calves are more robust .The ages were determined by questioning the patient's guardian and farmers.

**Demographic data and sample collection**

Samples were obtained from the rectum of calves using gloved fingers and were placed in sterile containers with screw caps. A stool specimen weighing between five and ten grams was collected. Preventive measures were implemented to minimize the risk of cross-contamination between specimens. To avoid direct contact with feces and any potential contaminants, gloves were used when doing this. To protect from contamination with clothing, lab coats or disposable gowns, and wear masks were used if there is in case of a possibility of splashing. We collected samples using sterile equipment and containers to prevent cross-contamination. Used clean, disposable containers made exclusively for stool collecting. Ensure that they were properly sealed to prevent leakage. The samples were promptly moved to a container with an ice bag and kept at a temperature of -20°C. In this cross-sectional study, during the period from February 2019 to June 2022, a standard pre-tested questionnaire was used to collect data on different demographic and epidemiological variables such as age, gender, clinical manifestations, symptoms, and date of specimen collection were documented.

**Identification by microscopy**

Before molecular testing, feces were examined microscopically for *Cryptosporidium* oocysts and *Giardia* cysts. All samples were stained with modified Ziehl-Neelsen acid-fast (Modified Z-N stain) (Putt, 1951) for *Cryptosporidia* and Trichrome stain (Siwila, 2017) for *Giardia* to detect the presence of *Cryptosporidium* oocysts and *Giardia* cysts, respectively. The microscopic slides were then examined with a Leica DM750 Binocular (Wetzlar, Germany) to confirm their presence. The cysts and oocysts of parasites were identified using the microscope with a magnification of 10x and 40x.

**DNA extraction and PCR analysis**

Genomic DNA from the whole stool samples were extracted by PureLink™ Microbiome DNA Purification Kit (Catalog Number A29790) following the manufacturer's instruction. The extracted DNA samples were then subjected to nested polymerase chain reaction (PCR) analysis for molecular-based identification. The PCR-based identification of *Cryptosporidium* and *Giardia* was carried out by a portion of the *SSU* gene (240 bp) (Nolan et al., 2013) and the *tpi* gene (~530) (Sulaiman et al., 2003), respectively. Therefore, the primary amplification of *SSU* was performed by using primers XF2 (forward: 5'-GGAAGGG-TTGTATTTATTAGATAAAG-3') and XR2 (reverse: 5'-AAGGAGTAAGGAACAACCTC-CA-3') (Koehler et al., 2016), followed by nested amplification of *SSU* using the internal primers pSSUf (forward: 5'-AAAGCTCGT-AGTTGGATTTCTGTT-3') and pSSUr (reverse: 5'-ACCTCTGACTGTTAAATACRA-ATG C-3') (Nolan et al., 2010). For the primary amplification, a cycling protocol of 94°C for 5min (initial denaturation), followed by 30 cycles of 94°C for 45 s (denaturation), 45°C for 2 min (annealing), and 72°C for 1.5min (extension), with a final extension of 72°C for 10 min was employed. At the same time, the secondary amplification was achieved by employing a cycling protocol of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension of 72°C for 10 min (Koehler et al., 2016).

The *tpi* locus was amplified using primers AL3543 (forward: 5'-AAATTATGCCTGCTC-GTCG-3') and AL3546 (reverse: 5'-CAAACCT-TTTCCGCAAACC-3'), followed by a nested

amplification of *tpi* employing primers AL3544 (forward: 5'-CCCTTCATCGGTGGTAACTT-3') and AL3545 (reverse: 5'-GTGGCCACCAC-TCCCGTGCC-3') (Sulaiman et al., 2003). For the primary amplification, the cycling protocol was 94°C for 5 min (initial denaturation), followed by 30 cycles of 94°C for 45 s (denaturation), 50°C for 45 s (annealing), and 72°C for 1 min (extension) and a final extension of 72°C for 10 min. The secondary amplification of *tpi* was achieved employing 94°C for 5 min, followed by 30 cycles of 94°C for 45s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min (Koehler et al., 2016).

### Statistical analysis

Descriptive and analytic statistics were performed in STATA (2013) after the data was input in Microsoft Excel 2019. The Chi-square test was used to assess and compare *Cryptosporidium* and *Giardia* infection rates with different variables in both children and calves. The graphical demonstration of the data was carried out by Microsoft Excel 2019. Values of $p<0.05$ were considered statistically significant.

### Ethics statement

This study was reviewed and approved by the Chattogram Veterinary and Animal Sciences University Ethics Committee. The CVASU Ethics Committee approval number for the project is Memo no.-CVASU/Dir(R&E) EC/2019/39(2/10). Written informed consent was obtained from the owners for the participation of both children and animals in this study.

## 3. RESULTS

A total of 437 fecal specimens were examined to determine the prevalence of *Cryptosporidium* and *Giardia* infection within human and animal populations in Bangladesh. Therefore, 200 human fecal samples of symptomatic children and 237 calve samples were collected from local hospitals and farms of Chattogram respectively. According to the modified Z-N stain, the prevalence of *Cryptosporidium* infection among hospitalized diarrheic children and infected calves was 13.5% and 23.63%, respectively (Figure 1). However, in *SSU* gene-based PCR, the prevalence of *Cryptosporidium hominis* infection among hospitalized diarrheic children and infected calves was 9.5% and 19.41%, respectively (Figure 1). Additionally, Trichome staining revealed that the prevalence of *Giardia* in children and calves was 9.5% and 19.41%, respectively. While *tpi* gene-based PCR showed a 9% *Giardia intestinalis* infection prevalence in children and 10.55% in calves (Figure 1).

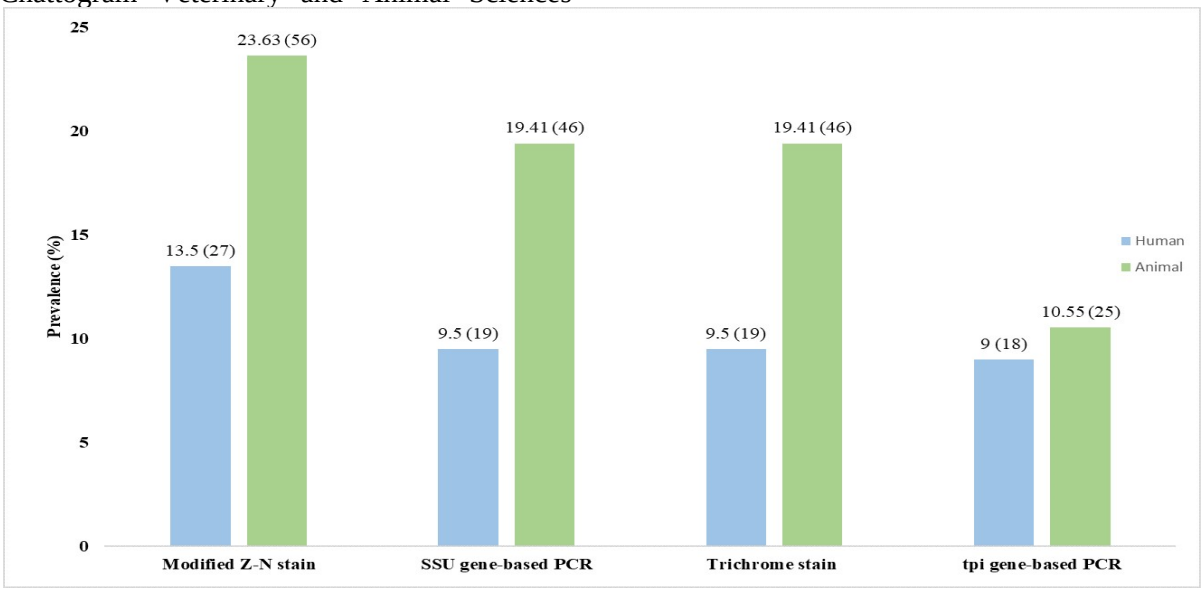


Figure 1. The prevalence of cryptosporidiosis and giardiasis in human and animal populations.

Table 1 summaries the descriptive statistics and associations between various characteristics and occurrences of cryptosporidiosis and giardiasis in the human population, as identified by the PCR method. In humans, gender-based distributions for cryptosporidiosis and giardiasis indicated that males 14.29% in cryptosporidiosis and 13.33% in giardiasis) were at a higher risk for infection than females (10.53% in cryptosporidiosis and 12.63% in giardiasis), without achieving statistical significance ($p$>0.005). Cryptosporidiosis was more prevalent in children aged 11-15 months, whereas giardiasis was more frequent in children more than >35 months. The variation of seasons significantly influences the prevalence and incidence of cryptos-poridiosis and giardiasis in the human population. Giardiasis 15.49% was shown to be more likely to spread during the winter months, while cryptos-poridiosis 14.81% was more predominant in summer. However, no statistically significant differences (p>0.005) were found across the seasons. In all scenarios, children residing in rural areas were more susceptible to infection (14.58% in cryptosporidiosis and 13.54% in giardiasis) than their counterparts residing in urban areas, including city and slum environments. Additionally, children who deprived with proper sanitation were more susceptible to both kind of infections (17.07% in cryptosporidiosis and 15.85% in giardiasis). Water sources were also impacted on both kind of infections, where children who consumed pond water rather than supplied and tube well water, were more prone to cryptosporidiosis 14.29% and giardiasis 42.86%. Drinking pond water is often associated with higher risks of waterborne diseases, including those caused by protozoa like *Cryptosporidium* and *Giardia*, due to potential contamination with pathogens. Nevertheless, no statistically significant (p>0.05) associations were identified in both circumstances. Children with a history of being mix (breastfed and formula) are more susceptible to both types of infections, where in case of giardiasis significant differences were recorded (p>0.05).

Table 1. Descriptive statistics and association of different variables with cryptosporidiosis and giardiasis in the human population diagnosed by PCR

| **Variable** | **Level** | **Total observation (%)** | **No. positive for *Cryptosporidium* (%)** | ***p-value* for $\chi^2$ test** | **No. positive for *Giardia* (%)** | ***p-value* for $\chi^2$ test** |
|---|---|---|---|---|---|---|
| Gender | Female | 95 (47.50) | 10 (10.53) | 0.42 | 12 (12.63) | 0.88 |
| | Male | 105 (52.50) | 15 (14.29) | | 14 (13.33) | |
| Age (months) | 1 to 10 | 55 (27.50) | 7 (12.73) | 0.24 | 6 (10.19) | 0.85 |
| | 11 to 15 | 45 (22.50) | 9 (20.00) | | 5 (11.11) | |
| | 16 to 35 | 50 (25.00) | 6 (12.00) | | 7 (14.00) | |
| | >36 | 50 (25.00) | 3 (6.00) | | 8 (16.00) | |
| Season of sample collection | Summer | 54 (27.55) | 8 (14.81) | 0.59 | 3 (5.56) | 0.06 |
| | Winter | 142 (72.45) | 17 (11.97) | | 22 (15.49) | |
| Residence | City | 76 (38.00) | 9 (11.84) | 0.56 | 10 (13.16) | 0.92 |
| | Slum | 28 (14.00) | 2 (7.14) | | 3 (10.71) | |
| | Village | 96 (48.00) | 14 (14.58) | | 13 (13.54) | |
| Source of water | Pond | 8 (4.00) | 1 (14.29) | 0.73 | 3 (42.86) | 0.06 |
| | Supply | 98 (49.25) | 10 (10.20) | | 11 (11.22) | |
| | Tube well | 94 (47.24) | 13 (13.83) | | 12 (12.77) | |
| History of breastfeeding | Yes | 115 (57.50) | 14 (12.17) | 0.55 | 14 (12.17) | 0.02 |
| | No | 82 (41.00) | 10 (12.20) | | 10 (12.20) | |
| | Mix | 3 (1.50) | 1 (33.33) | | 2 (66.67) | |
| History of pet | Yes | 96 (48.00) | 14 (14.58) | 0.39 | 10 (10.42) | 0.30 |
| | No | 104 (52.00) | 11 (10.58) | | 16 (15.38) | |
| History of anthelmintics | Yes | 57 (28.50) | 5 (8.77) | 0.31 | 5 (8.77) | 0.26 |
| | No | 143 (71.50) | 20 (13.99) | | 21 (14.69) | |

The correlations between clinical characteristics and disease prevalence were also investigated and analyzed. The condition was not shown to be associated with a history of nausea, abdominal pain, anorexia, vomiting, or dehydration (Table 2). In case of calves the frequency of cryptosporidiosis (13.19%) and giardiasis (2.78%) was reported higher in females in comparison to males (10.75% and 2.15% respectively) although the association had no significant differences (p>0.05). The frequency of cryptosporidiosis was found to be relatively high in calves aged between 31 and 60 days; and in case of *Giardia* it was higher in calves aged between 16-31 days, though the association was not significant across the age groups (p>0.05). At the farm level, the frequency of cryptosporidiosis was shown to be independent of many factors, including the education level of owners, topography, types of calf housing, and floor type (p>0.05) (Table 4). Nevertheless, a substantial prevalence of cryptosporidiosis was seen in calves residing in hilly regions (17.65%) and giardiasis was seen 5.88%. Furthermore, it was shown that the incidence of cryptosporidiosis was comparatively elevated in calves that were exposed to groundwater 15.87% as opposed to pond 0.00% or supply water 11.54% and also in giardiasis it was high in ground water sources. However, no statistically significant difference was evident between these variables (p>0.05).

A farm's drainage system appeared not to have any effect on cryptosporidiosis and giardiasis prevalence. Notably, calves that had fortnightly bedding changes had a higher frequency (17.39 %) of cryptosporidiosis but not in giardiasis (2.17%) although this difference was not statistically significant (p>0.05). There was also an increase in the frequency (28%) of cryptosporidiosis among calves that did not share feeding utensils but not in giardiasis. Moreover, the frequency of cryptosporidiosis was (66.67%) higher in calves that were hand-fed compared to suckling-fed (13.56%). And whereas in case for gardiasis the prevalence was relatively much (33.33%) higher in hand fed calves compared to suckler calves (2.82%). However in case of giardiasis there were significant differences (p>0.05) in comparison to between the two feeding methods (Table 4).

Table 2. Description of clinical findings in cryptosporidiosis and giardiasis in the human population diagnosed by PCR

| **Clinical findings** | **Level** | **Total observation (%)** | **No. positive for *Cryptosporidium* (%)** | ***p-value* for $\chi^2$ test** | **No. positive for *Giardia* (%)** | ***p-value* for $\chi^2$ test** |
|---|---|---|---|---|---|---|
| History of nausea | Yes | 68 (34) | 6 (8.82) | 0.26 | 8 (11.76) | 0.71 |
| | No | 132 (66) | 19 (14.39) | | 18 (13.64) | |
| Abdominal discomfort | Yes | 76 (38.00) | 10 (13.16) | 0.82 | 7 (9.21) | 0.21 |
| | No | 124 (62.00) | 15 (12.10) | | 19 (15.32) | |
| Anorexia | Yes | 15 (7.50) | 2 (13.33) | 0.91 | 2 (13.33) | 0.97 |
| | No | 185 (92.50) | 23 (12.43) | | 24 (12.97) | |
| Vomiting | Yes | 68 (34.0) | 13 (19.12) | 0.04 | 11 (16.18) | 0.34 |
| | No | 132 (66.0) | 12 (9.09) | | 15 (11.36) | |
| Dehydration | Yes | 103 (51.50) | 15 (14.56) | 0.36 | 15 (14.56) | 0.50 |
| | No | 97 (48.50) | 10 (10.31) | | 11 (11.34) | |

## 4. DISCUSSION

*Cryptosporidium* and *giardia* have the potential to cause clinical disease in calves and to be transmitted to other animal species and humans. Detection of *Cryptosporidium* and *Giardia* in children and calves therefore may be of great public health significance, as humans and animals share sometimes the same premises in rural settings. Based on the findings of the modified Z-N stain, the prevalence of *Cryptosporidium* infection was determined to be 13.5% among hospitalized children, whereas infected calves exhibited the prevalence of 23.63%. However, the *SSU* gene-based PCR method revealed that the frequency of *Cryptosporidium* infection was 9.5% among hospitalized children with diarrhea and 19.41% among infected calves.

Table 3. Descriptive statistics and association of different variables with *Cryptosporidium* and *Giardia* at the animal level diagnosed by PCR

| Variable | Level | Total observation (%) | No. positive for *Cryptosporidium* (%) | *p-value* for $\chi^2$ test | No. positive for *Giardia* (%) | *p-value* for $\chi^2$ test |
|---|---|---|---|---|---|---|
| **Sex** | Female | 144 (60.76) | 19 (13.19) | 0.58 | **4 (2.78)** | |
| | Male | 93 (39.24) | 10 (10.75) | | **2 (2.15)** | 0.77 |
| **Age (days)** | 1 to 15 | 49 (20.68) | 6 (12.24) | 0.35 | 1 (2.04)) | |
| | 16 to 30 | 58 (24.47) | 7 (12.07) | | **3 (5.17)** | |
| | 31 to 60 | 70 (29.54) | 12 (17.14) | | 0 | 0.30 |
| | >60 | 60 (25.32) | 4 (6.67) | | 2 (3.33) | |

This study showed a prevalence of 13.5% for *Cryptosporidium* in children with persistent diarrhea diagnosed by the modified Ziehl–Neelsen staining method, where this percentage agrees with the results of (Tairsh et al., 2017) who reported the prevalence of 12.85%. However, the previous study by Ehsan et al. (2015) suggested that the prevalence of the *SSU* gene was found to be 5% each in calves infected with *Cryptosporidium* infection. While the prevalence of 1-4% was reported in Europe and North America, and it was 3-20% in Australia, Asia, Africa, and Central and South America (WHO, 2009). Another study (Morgan et al., 1998) reported 36% by microscopic and 29% by PCR method. Furthermore, Trichrome staining techniques indicated that the occurrence of *Giardia* in children and calves was 9.5% and 19.41%, respectively. The findings are similar to the previous study reported by Datta (2024). In contrast the previous study by Ehsan et al. (2015) reported that the prevalence of the *tpi* gene was found to be 21.7% in calves infected with *Giardia* infection. In this study, *tpi gene*-based PCR analysis revealed a prevalence rate of 9% for *Giardia* infection in children and 10.55% in calves hence the results were in agreement with (Alseady et al., 2023). Usually the PCR is substantially more sensitive than staining methods. However, the outcomes of the study for cryptosporidiosis and giardiasis staining results were higher than the PCR result.

This difference may be due to sample size and diagnostic techniques because PCR may yield less accurate results than microscopy due to issues with sample preparation or PCR inhibitors, microscopy can directly visualize parasites regardless of these conditions. Furthermore, PCR approaches may not target all parasite strains or stages, resulting in different detection results.

Cryptosporidiosis and giardiasis are gastro-intestinal diseases caused by parasitic protozoa such *Cryptosporidium* and *Giardia*, respectively (Ehsan et al., 2015).Understanding the factors that influence the prevalence of these infections is essential for public health efforts. However, the present study delves into gender-based distributions, age, season, urban-rural residence, water sources, breastfeeding, and pet ownership/exposure to anthelmintics, analyzing their potential impact on the prevalence of cryptosporidiosis and giardiasis in the human population. The data suggests a slight gender-based difference in infection rates, with males showing a marginally higher risk for both cryptosporidiosis 14.29% and giardiasis 13.33% compared to females 10.53% and 12.63 %, respectively. However, these differences were not statistically significant ($p>0.05$). It is important to note that gender alone may not be a primary determinant of infection risk, and other factors may play a more significant role. Regarding giardiasis, the findings are similar to the previous study by (Suman et al., 2011). Additionally, research conducted in India and Nigeria has shown a higher prevalence of giardiasis infection in males than females (Dwivedi et al., 2007). The data also indicates that cryptosporidiosis is more prevalent in children aged 11-15 months, whereas giardiasis is more frequent in children older than 35 months. The prevalence of giardiasis is analogous to other previous studies by Haque et al. (2003) and Suman et al. (2011).

Table 4. Descriptive statistics and association of different variables with *Cryptosporidium* and *Giardia* at farm level diagnosed by PCR

| **Variable** | **Level** | **Total observation (%)** | **No. positive for *Cryptosporidium* (%)** | ***p- value* for $\chi^2$ test** | **No. positive for *Giardia* (%)** | ***p-value* for $\chi^2$ test** |
|---|---|---|---|---|---|---|
| Owners' education | Illiterate | 7 (3.93) | 0 | 0.16 | 0 | |
| | Primary | 24 (13.48) | 3 (12.50) | | 0 | |
| | Secondary | 42 (23.60) | 7 (16.67) | | 2(4.76) | |
| | Higher Secondary | 32 (17.98) | 8 (25.00) | | 1(3.13) | 0.55 |
| | Graduation | 44 (24.58) | 3 (6.82) | | 3(6.82) | |
| | Post-graduation | 30 (16.85) | 5 (16.67) | | 0 | |
| Topography | Hilly | 34 (18.89) | 6 (17.65) | 0.55 | 4(5.88) | 0.35 |
| | Plain land | 146 (81.11) | 20 (13.70) | | 2 (2.47) | |
| Types of calf house | Closed barn | 116 (64.44) | 17 (14.66) | 0.99 | 4 (3.45) | |
| | Open barn | 15 (8.33) | 2 (13.33) | | 0 | 0.74 |
| | Partial open | 49 (27.22) | 7 (14.29) | | 2 (4.08) | |
| Floor-type | Brick | 92 (51.11) | 13 (14.13) | 0.97 | 3 (3.26) | |
| | Concrete | 73 (40.56) | 11 (15.07) | | 1 (1.37) | 0.06 |
| | Muddy/jute/wood | 15 (8.33) | 2 (13.33) | | 2 (13.33) | |
| Source of drinking water | Ground | 126 (70.0) | 20 (15.87) | 0.64 | 4 (3.17) | |
| | Pond or stream | 2 (1.11) | 0 | | 0 | 0.94 |
| | Supply | 52 (28.89) | 6 (11.54) | | 2 (3.85) | |
| Type of drainage system | None | 32 (17.78) | 5 (15.63) | 0.75 | 2 (6.25) | |
| | Open | 118 (65.56) | 18 (15.25) | | 3 (2.54) | 0.59 |
| | Sub-surface | 30 (16.67) | 3 (10.00) | | 1 (3.33) | |
| Frequency of bedding change | Never | 18 (10.0) | 2 (11.11) | 0.72 | 1 (5.56) | |
| | Weekly | 111 (61.67) | 16 (14.41) | | 3 (2.70) | |
| | Fortnightly | 46 (25.56) | 8 (17.39) | | 1 (2.17) | 0.18 |
| | Monthly | 5 (2.78) | 0 | | 1 (20.00) | |
| Hygiene of calf feeding utensils | Not shared | 25 (13.89) | 7 (28.0) | 0.11 | 1 (4.00) | |
| | Shared and disinfected | 45 (25.0) | 6 (13.33) | | 0 | 0.35 |
| | Shared and washed with water | 110 (61.11) | 13 (11.82) | | 5 (4.55) | |
| Feeding of milk | Hand feeding | 3 (1.67) | 2 (66.67) | 0.01 | 1 (33.33) | |
| | Suckling | 177 (98.33) | 24 (13.56) | | 5 (2.82) | 0.004* |

Cryptosporidiosis peaks in the 11-15 month age group possibly due to their developing immune systems and increased exposure to infection through behaviors like mouthing objects and interacting in group settings. However, the potential factors contributing to this age-dependent trend are likely associated with the behaviors of children. Another potential factor contributing to the increased incidence of infections in children might be the insufficient development of efficient immunity (Suman et al., 2011). However, no statistically significant differences were found. This suggests that while age may contribute to susceptibility, it is not a sole determinant of infection risk. Seasonal variation substantially impacts the prevalence and incidence of cryptosporidiosis and giardiasis, where the summer season was found to be more feasible for *Cryptosporidium* and winter for *Giardia* infections. Giardiasis is more prevalent in the winter possibly because milder temperatures preserve *Giardia* cysts in the environment and increase indoor activity, whereas cryptosporidiosis is more common in the summer because warmer temperatures and increased recreational water use encourage the survival and spread of *Cryptosporidium* oocysts. Children residing in rural areas appear to be

more susceptible to infection (14.58% in cryptosporidiosis and 13.54% in giardiasis) than their urban counterparts. However, the lack of statistical significance ($p>0.05$) suggests that while residence may play a role, other factors are at play, too, including access to healthcare and sanitation facilities. Although the lowest prevalence of both the protozoa among the slum dwellers usually ideal factors for the development but in some areas, targeted public health interventions may have improved water quality, sanitation, and hygiene practices, even in slum environments. These improvements can significantly reduce the prevalence of protozoan infections and also residents in slums may develop partial immunity due to repeated low-level exposures could lead to lower reported prevalence rates.

Notably, children lack of proper sanitation facilities was more susceptible to both kind infections. *Cryptosporidium* and *Giardia* are often spread by contaminated water; hence, it was interesting to see whether the water source affected the likelihood of infection. In this study, no statistically association was seen in the water source, though, children who had consumed pond water, were more prone to cryptosporidiosis and giardiasis. Two other studies conducted in urban and semi-urban regions in Brazil (Pereira et al., 2002) and Guinea Bissau (Mølbak et al., 1994) have also similarly shown no significant correlation between cryptosporidiosis and either the source or type of water supply. Children who had previously been nursed and formula fed were shown to be more susceptible to both types of illnesses, but there were significant differences ($p>0.05$) in giardiasis. Children with a history of mix feeding have a relatively high prevalence of *Cryptosporidium* and *Giardia* infection which could be attributed to supplemental feeding and difficulty with exclusive breastfeeding.

According to this study, cryptosporidiosis was more prevalent in calves than children. The present study also showed that cryptosporidiosis was expected in 31–60-day-old calves, which is supported by other previous studies by (Gow and Waldner, 2006; Khair et al., 2014; Maldonado-Camargo et al., 1998; Paul et al., 2009); and (Swai and Schoonman, 2010). The increased incidence of *cryptosporidium* and *giardia* in calves aged 16 to 30 days could be attributed to increased exposure to contaminated environment when their immune systems are still maturing. This age range frequently coincides with the period during which calves are more active and contact with polluted feed or water sources. As a fore mentioned, the prevalence of cryptosporidiosis in females was high (13.19%), which is opposite to the study conducted by (Nouri and Toroghi, 1991), it was shown that male diarrheic calves had a greater incidence of infection compared to female calves (Nouri and Toroghi, 1991). However, the prevalence of cryptosporidiosis did not exhibit a statistically significant difference based on the gender of calves, which is consistent with findings reported by previous studies of (Khair et al., 2014; Rahaman et al., 1984).

## 5. CONCLUSION

The study aims to inform public health strategies and improve infection control measures for both humans and animals. Examining diarrhoeic samples from both children and calves in the same city for *Cryptosporidium* and *Giardia* aims to highlight the zoonotic importance of these parasites. By investigating infections in both human and animal populations, the study seeks to demonstrate how these parasites can be transmitted between species and affect both groups. This approach underscores the significance of zoonotic transmission and helps in understanding the broader implications for public health and animal management. Regarding this study, the epidemiology of cryptosporidiosis and giardiasis in Bangladesh indicates a complex, challenging context. Therefore, the epidemiology of cryptos-poridiosis and giardiasis in Bangladesh highlights the urgent need for extensive public health efforts to combat these enduring and disabling parasitic illnesses. The well-being and contentment of the people will improve, and the entire country will move closer to its long-term aim of sustainable development if this objective is met.

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