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Prospective Cohort Study of *Cryptosporidium* Infection and Shedding in Infants and Their Households

Poonum Korpe,[1] Zhanmo Ni,[1] Mamun Kabir,[2] Masud Alam,[2] Tahsin Ferdous,[2] Rifat Ara,[2] Rebecca M. Munday,[3] Rashidul Haque,[2] and Priya Duggal[1]

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; [2]Emerging Infections and Parasitology Laboratory, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh; and [3]Department of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Background. *Cryptosporidium* spp. are responsible for significant diarrheal morbidity and mortality in under-5 children. There is no vaccine; thus, a focus on prevention is paramount. Prior studies suggest that person-to-person spread may be an important pathway for transmission to young children. Here we describe a longitudinal cohort study of 100 families with infants to determine rates of cryptosporidiosis within households during the coronavirus disease 2019 (COVID-19) pandemic.

Methods. Families living in Mirpur, Bangladesh, with 1 infant aged 6–8 months were enrolled and followed with weekly illness survey and stool testing for *Cryptosporidium* for 8 months.

Results. From December 2020 to August 2021, 100 families were enrolled. Forty-four percent of index children and 35% of siblings had at least 1 *Cryptosporidium* infection. Shedding of *Cryptosporidium* occurred for a mean (standard deviation) of 19 (8.3) days in index infants, 16.1 (11.6) days in children 1–5 years, and 16.2 (12.8) days in adults. A longer duration of *Cryptosporidium* shedding was associated with growth faltering in infants. There was a spike in *Cryptosporidium* cases in May 2021, which coincided with a spike in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases in the region.

Conclusions. In this intensive, longitudinal study of *Cryptosporidium* infection in families we found high rates of cryptosporidiosis in infants and children, and prolonged parasite shedding, especially among malnourished children. These data support that transmission within the household is an important route of exposure for young infants and that treatment of nondiarrheal infection to interrupt person-to-person transmission within the home may be essential for preventing cryptosporidiosis in infants.

Keywords. *Cryptosporidium*; Bangladesh; diarrheal disease.

Cryptosporidium, an enteric protozoa, has been identified as a leading contributor to moderate-to-severe diarrhea in children younger than 2 years of age worldwide [1]. *Cryptosporidium* diarrhea and subclinical infection have both been associated with stunted growth and cognitive deficits in children [2–5]. Despite the high morbidity from this infection, there is currently no effective treatment for infants younger than 1 year of age.

Cryptosporidium is transmitted by fecal-oral contamination. Transmission of diarrheal pathogens has been traditionally described as occurring along the “five-F pathways”: fluids, fingers, food, fields, and flies [6]. *Cryptosporidium* oocysts are readily infectious when excreted in feces, and ingestion of just 10 oocysts can result in infection [7]. *Cryptosporidium* is also resistant to chlorination [8, 9]. Sporadic waterborne outbreaks have been well documented in industrialized countries, but in endemic areas, like Bangladesh, infection is associated with poor sanitation, poverty, animal rearing, and malnutrition [3, 10, 11].

Efforts to combat *Cryptosporidium* infection include improving the quality of drinking water and sanitation. However, nonpharmacologic studies to reduce environmental exposure and contamination have not been successful at preventing symptomatic or asymptomatic infections. In a semiurban slum in India, where cryptosporidiosis is endemic, an intervention aimed at providing clean water to households found that children who drank bottled water did not have reduced rates of *Cryptosporidium* infection compared with those who drank from the municipal water supply [12]. Moreover, a community-based introduction of membrane-filtered drinking water, with pore size small enough to filter *Cryptosporidium* spp., did produce microbiologically safe drinking water for 1 year; however, this did not result in reduced rates of diarrhea in children under 2 years [13]. The Water quality, Sanitation, and Handwashing (WASH) Benefits study, a cluster-randomized controlled trial, demonstrated success in providing clean water and improved handwashing and sanitation in communities in rural Bangladesh; however, despite these improved measures, there was no improvement in the rate of cryptosporidiosis and there was no significant improvement in child growth [14]. This study highlighted that

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Correspondence: P. Korpe, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St, E6031, Baltimore, MD 21205 (pkorpe1@jhu.edu).

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cryptosporidiosis may not be amenable to traditional WASH interventions because of its low infectious dose.

In a small pilot study, we previously demonstrated that 39% of households with a *Cryptosporidium*-infected child had a second infected household member. Furthermore, genotyping of Cryptosporidia suggested that transmission in young children primarily occurs via human-to-human transmission rather than zoonosis and emphasized the importance of household transmission in *Cryptosporidium* infections for children under 2 years of age [15].

To further explore *Cryptosporidium* transmission, we enrolled a cohort of 100 families with an index child 6–8 months of age and followed them prospectively for 8 months. The extensive longitudinal follow-up provides an opportunity to define the incidence of *Cryptosporidium* infection within families with infants and to identify factors involved in transmission of the disease. Enrollment began during the early years of the coronavirus disease 2019 (COVID-19) pandemic, allowing a unique perspective on the effect of social-distancing measures on transmission of an enteric protozoal infection.

METHODS

Study Design

Families with infants 6 to 8 months old living in Mirpur, Bangladesh, were identified and approached for enrollment into the study. If families agreed to participate, all household members, defined as any individual sleeping under the same roof or eating from the same cooking pot, were consented for enrollment. Once enrolled, a demographic enrollment form was completed for each participant. Baseline characteristics collected included age, weight, height, and familial relationships from all participants. At the household level, socioeconomic data including household income, parental occupation, maternal education, and crowding were collected. Additionally, data on water, sanitation, and household environment were collected, including source of drinking water, method of treatment of drinking water, toilet type, and presence of domesticated animals in the home.

Subsequently, all index infants and family members were surveyed weekly for any illness, including diarrhea and respiratory illness. A stool specimen was collected from each participant weekly, over an 8-month follow-up period. If a participant was experiencing diarrhea, a sample was collected, and this was counted as that week's stool collection. Stool was not collected more frequently than once a week. A serum specimen was collected from participants at baseline and at the end of the 8-month follow-up.

Laboratory Testing

Stool and serum samples were collected in Mirpur and transported via cold chain to the Parasitology Laboratory at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,b). Testing for *Cryptosporidium* was performed using the *Cryptosporidium* II enzyme-linked immunosorbent assay (ELISA) kit (TechLab, Blacksburg, VA) on all weekly and diarrheal stool samples. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G (IgG) testing was performed using the TechLab SARS-CoV-2 ELISA (investigational use only) on baseline and 8-month serum samples collected from each participant.

Case Definitions and Statistical Analysis

Symptoms were analyzed based on the weekly clinical survey of each participant. A *Cryptosporidium* case was defined as a participant having at least 1 stool sample positive for *Cryptosporidium* during the 8-month follow-up period. A diarrheal episode was defined as 3 or more loose stools per day, recurring daily within a 14-day period. A diarrhea-associated positive *Cryptosporidium* episode was defined as at least 1 positive diarrhea sample during the episode of infection.

The duration of *Cryptosporidium* shedding was calculated for each episode of *Cryptosporidium* infection. An episode was defined as stool samples testing positive within a 14-day period. The duration of the episode was calculated by the difference in days between the first positive stool sample in the episode and the first negative stool sample after the episode. If a positive sample was collected in the last week of study follow-up, this was assigned a minimum 7-day duration of positivity.

The height-for-age adjusted *z* scores (HAZ) and weight-for-age adjusted *z* scores (WAZ) were calculated based on World Health Organization standards [14]. Lengths/heights and weights of the participants were measured monthly after baseline enrollment. The ages of children were rounded to the nearest month at the time of each measurement.

Socioeconomic Definitions

"Overcrowding" in the home was classified as more than 3 people per room per household [16]. Drinking water was categorized as water piped into the home, water piped into the yard, and tube well [17]. "Unimproved" toilets were categorized as having no facility or traditional pit latrine without slab. "Improved" toilet included ventilated improved pit toilet with water seal and flush toilet to a piped sewer system, septic tank, or pit latrine [17].

Statistical Analysis

Linear regression was used to estimate the difference in duration of shedding between index children compared with other children compared with adults, adjusting for sex and baseline COVID-19 IgG result. Linear regression was also applied to identify risk factors associated with longer duration of shedding. Both models are presented, and models with the lowest Akaike information criterion (AIC) based on stepwise model

Table 1. Characteristics of Index Infants and Household Members at Enrollment

Index infants and household members, n	342
Index infants	100
Mothers	100
Fathers	49
Siblings	60
Grandmothers	15
Aunts	10
Uncles	2
Cousins	6
Age of index infants at enrollment, mean (SD), d	226.8 (26.7)
Female index infants, %	46
Index infants' height-for-age adjusted *z* score at enrollment, mean (SD)	−1.15 (1.16)
Index infants" weight-for-age adjusted *z* score at enrollment, mean (SD)	−0.74 (1.03)
No. of individuals per household, median (IQR)	5 (4, 6)
Household income in BDT,[a] median (IQR)	15 000 (10 000, 20 000)
Educational level of the mother, %	
No formal education	17
Some primary school	36
Some high school	36
Secondary school diploma	15
Households with overcrowding, %	48
Households with domesticated animals, %	16[a]
Water source, %	
Piped into home	85
Piped into yard	2
Deep tube well	15
Method of water treatment, %	
None	30
Boiling	64
Filter	6
Toilet type, %	
Improved	
Ventilated improved pit water seal	72
Flush toilet	1
Unimproved	
Traditional pit toilet	27
Households sharing toilet, %	98
If sharing, number of other households sharing toilet, mean (SD)	2.2 (1.56)

Abbreviations: BDT, Bangladeshi Taka; IQR, interquartile range; SD, standard deviation.
[a]Chicken or ducks were kept at 100% of the households that had any animals.

selection. Logistic regression was used to identify risk factors for *Cryptosporidium*-positive cases at the household level. All tests were based on a 2-sided $P < .05$. Fisher's exact test was used for comparing group differences of categorical variables and Wilcoxon-Mann-Whitney test was used for continuous variables in exploratory analysis because of their relaxed assumptions on large sample sizes. Chi-square tests were used to compare difference in symptoms reported among weeks with *Cryptosporidium* infection and negative for infection among participants. Analysis was performed using R, version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

Ethics Approval

The study was approved by the Research and Ethics Review Committees at the ICDDR,b and by the Institutional Review Board at the Johns Hopkins University Bloomberg School of Public Health. Written consent or assent was obtained from all participants over 11 years old, and parental consent was obtained for all children 11 years old and younger.

RESULTS

From 29 September 2020 to 22 December 2020, field workers conducted a census in Mirpur wards 2, 3, and 5 and identified 1841 households. Of these, 284 families were identified as being eligible and were screened for enrollment. Between December 2020 and August 2021, 100 families were enrolled into the study, with a mean duration of follow-up of 212.7 days (standard deviation [SD]: 28.8). Of the 100 families enrolled, 4 families were lost to follow-up and had a mean duration of follow-up of 98.6 days (SD: 54.2).

Families were composed of 100 index children between the ages of 6 and 8 months and 242 family members (Table 1) (total n = 342), including 100 mothers, 49 fathers, and 60 siblings. Sociodemographic characteristics of this cohort were similar to prior studies in this area [3, 15, 18]. Seventeen percent of primary caregivers had no formal education. Overcrowding was found in 48% of households. Sixteen percent of households owned animals, and these were chickens or ducks. Nearly one-third of households had an unimproved toilet, which includes

Table 2. *Cryptosporidium*-Positive Diarrheal and Surveillance Stools Collected During 8 Months of Follow-up, Listed by Age Group

	Diarrheal Stools, *Cryptosporidium* Positive, n (%)	Surveillance Stools, *Cryptosporidium* Positive, n (%)	Total Stools, *Cryptosporidium* Positive, n (%)
Index children	23 (7.6%)	106 (3.9%)	129 (4.2%)
Children, 1 to 5 y	2 (20%)	20 (2.8%)	22 (3.0%)
Children, 5–10 y	0 (0%)	5 (0.5%)	5 (0.5%)
Children, 10–17 y	0 (0%)	3 (0.8%)	3 (0.8%)
Adults	1 (2%)	16 (0.3%)	17 (0.3%)
All	26 (6.8%)	150 (1.5%)	176 (1.7%)

no toilet or a pit latrine. Treatment of drinking water was common and included boiling water prior to drinking (64%) or using a filter (6%).

In total, 9774 surveillance stool samples were collected, with a 1.5% incidence of *Cryptosporidium* spp. positivity, and in the 384 diarrheal stool samples, *Cryptosporidium* positivity was 6.8%. Among index infants, *Cryptosporidium* positivity was 3.9% in surveillance stools and 7.6% in diarrheal stools (Table 2). Among the 100 index infants, there were 239 episodes of diarrhea. The mean duration of diarrhea was 7.6 days (SD: 9.6) (Table 3). Index infants had more episodes of diarrhea and longer duration of diarrhea than all other age groups.

Table 3. Duration of Diarrhea by Age Group

Age Group	No. of Episodes of Diarrhea	Mean (SD) Duration in Days
<1 y	239	7.6 (9.6)
1–5 y	11	3.4 (2.2)
6–10 y	13	5 (5.0)
11–17 y	8	3 (1.2)
≥17 y	54	2.9 (4.4)

Abbreviation: SD, standard deviation.

Table 4. Duration of *Cryptosporidium* Shedding by Age Group

Age Group	No. of Episodes of *Cryptosporidium* Infection	Mean (SD) Duration in Days
<1 y	47	19.9 (8.3)
1–5 y	10	16.1 (11.6)
6–10 y	5	7.2 (1.1)
11–17 y	3	7.0 (0)
≥17 y	8	16.2 (12.8)

Abbreviation: SD, standard deviation.

When we considered *Cryptosporidium* infections per participant, children under age 5 carried the greatest burden of *Cryptosporidium* infection. The infected individuals included 44% of index infants, 35% of 2–5-year-olds, 15% of 6–10-year-olds, and 20% of 11–17-year-olds with at least 1 *Cryptosporidium* infection during the 8-month follow-up period. Among adult caregivers, 6% of mothers and 2% of fathers tested positive for *Cryptosporidium*. In total, 8 out of 170 (5%) adults tested positive for *Cryptosporidium* at least once during the study.

Shedding of *Cryptosporidium* occurred, on average, for 19.9 days (SD: 8.3) among index infants (Table 4). Index infants had more prolonged shedding than other age groups (Figures 1 and 2). In the linear regression model, older children had a shorter duration of shedding compared with index infants (beta: −7.6; 95% confidence interval [CI]: −13, −2.4) (Supplementary Table 1). As expected, among index infants, we found that growth faltering (HAZ ≤ −2) was significantly associated

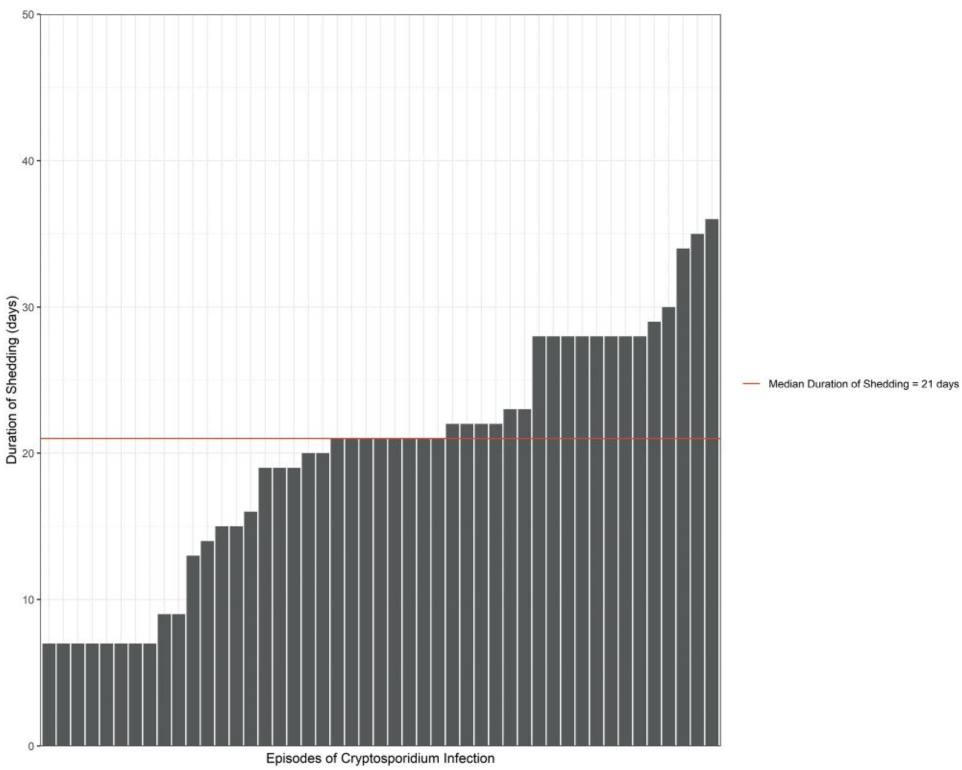

Figure 1. Duration of *Cryptosporidium* shedding among index children.

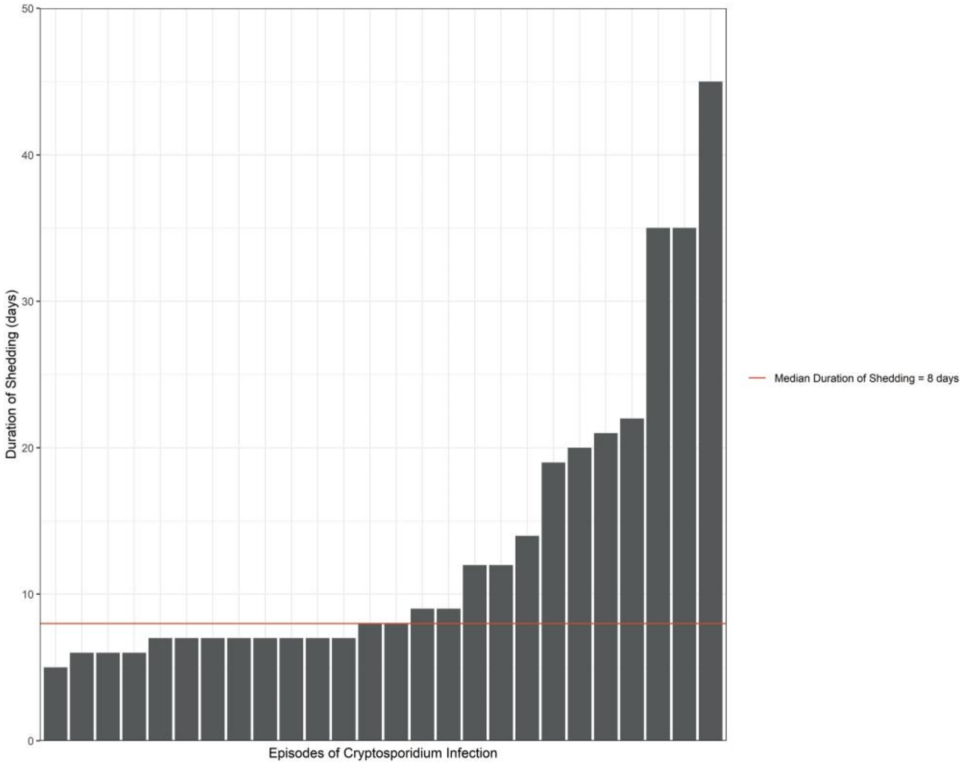

Figure 2. Duration of *Cryptosporidium* shedding among household members.

Table 5. Duration of *Cryptosporidium* Shedding in Individuals With Repeat Infections

Participant	Age, Y	Sex	Duration of Shedding in First Episode, D	Duration of Shedding in Second Episode, D
Index child	<1	Female	7	7
Index child	<1	Male	7	29
Index child	<1	Female	21	28
Sister	3	Female	7	20

with a longer duration of shedding (beta: 8.0; 95% CI: .41, 16) (Supplementary Table 2). The presence of animals in the home and treatment of drinking water were negatively associated with the duration of shedding in index children.

Repeat infections only occurred in young children. Three index infants, plus 1 unrelated sister, experienced repeat infections, and all were aged 3 years and younger (Table 5). Interestingly, 3 of the 4 children had a longer duration of parasite shedding during their second infection compared with their first infection.

Figure 3 shows weekly stool results among all 44 children who tested positive for *Cryptosporidium* in at least 1 weekly sample over the 8-month follow-up period. We observed that not all diarrheal episodes were positive for *Cryptosporidium*, and in some cases, a child may have had multiple diarrheal episodes and only a single weekly positive *Cryptosporidium* infection. And yet others had prolonged shedding spanning several weeks but no or limited diarrhea.

The monthly rate of *Cryptosporidium* positivity among stool samples was consistent throughout the study; however, there was a large increase in infections during May 2021 (Figure 4) [19]. There was a spike in COVID-19 cases in April 2020, after which the Bangladesh government mandated a strict lockdown. These restrictions were lifted in May 2020, which is when the corresponding spike in *Cryptosporidium* cases was observed [20]. Of all the enrolled participants ($n = 342$), 30.4% were SARS-CoV-2 IgG positive at baseline and this percentage increased to 45.8% at the end of the study. Eighty-nine participants went from a negative to positive SARS-CoV-2 serology during the 8-month follow-up period. SARS-CoV-2 IgG positivity in children was not associated with markers of malnutrition, and there were no observed differences in positivity by HAZ or height-for-weight adjusted z scores (t test, $P > .05$).

DISCUSSION

In this prospective, longitudinal study of infants and families living in an urban area in Bangladesh, there was a high rate of *Cryptosporidium* infection and shedding in young children with or without diarrhea. We also observed that household

Figure 3. Weekly stools of *Cryptosporidium*–positive index children aged over 8 months. Abbreviation: Crypto, *Cryptosporidium.*

family members had higher rates of infection, including 35% percent of children aged 2–5 years and 6% of mothers.

Shedding of *Cryptosporidium* lasted from 5 to 36 days among index infants. Although infection rates were lower in

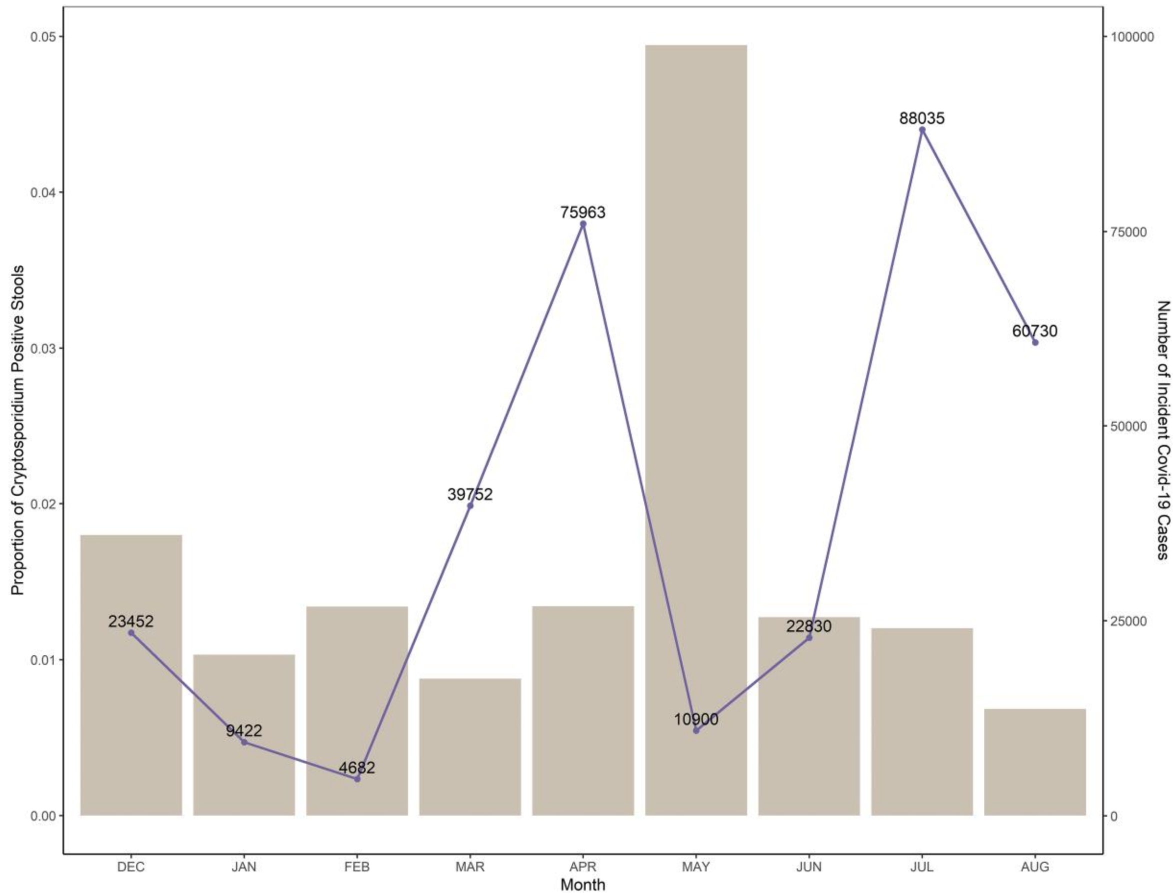

Figure 4. Proportion of positive stool samples by month from December 2020 to August 2021, with the number of monthly incident COVID-19 cases in Dhaka District. Abbreviation: COVID-19/Covid-19, coronavirus disease 2019.

older ages, prolonged shedding was observed across the age spectrum. Notably, even in adult participants, we found *Cryptosporidium* shedding to range from 5 to 35 days. We observed *Cryptosporidium* shedding in both diarrheal and nondiarrheal infections. Interestingly, growth faltering was associated with prolonged duration of shedding. The presence of domestic fowl in the home and treatment of drinking water were marginally inversely associated with duration of shedding. Treatment of drinking water may be directly related to decreased shedding by reducing the burden of parasite exposure through participants' gastrointestinal tracts. Alternatively, treatment of drinking water may be a proxy for other conditions that predispose to reduced parasite shedding.

The only other study that has reported on *Cryptosporidium* shedding with the same intensity as the present study was in Scotland in 1988, which looked at immunocompetent children and adults ages 9 months to 88 years. The Scottish study also reported a range of *Cryptosporidium* shedding from 2 to 35 days [21] using daily household follow-up of all *Cryptosporidium*-positive patients from their region. This differs from modeling-based projections, which have reported longer shedding times but may represent a lack of precision in those models [22].

We did not observe any reduced duration in shedding in second versus primary infections, suggesting that prior infection does not protect or prime for repeat episodes of shedding. A potential explanation for this could be that the primary and secondary infection involved distinct *Cryptosporidium* species. In gnotobiotic pigs, infection with *Cryptosporidium hominis* did not protect against infection from *Cryptosporidium parvum*, suggesting a lack of cross-species immunity [23]. The next phase of our study will further explore this question by sequencing all collected *Cryptosporidium* isolates.

The duration of oocyst shedding is important for transmission, as it represents the period of time an individual is infectious and can spread the infection to close contacts. The prolonged shedding observed in all age groups provides multiple opportunities for transmission within a household. In addition, we report shedding not only in the diarrheal episodes but also in surveillance samples. Efforts to treat or disrupt

Cryptosporidium infections that solely target diarrheal infections will not be adequate to control transmission.

Our data, combined with the Scottish data, demonstrate that, in a healthy population or in a malnourished population, *Cryptosporidium* shedding still occurs for a prolonged period, especially in children. Interventions to prevent *Cryptosporidium* infection in vulnerable populations must address the fact that shedding is occurring even when participants are not experiencing diarrhea. With this prolonged shedding in the household environment, and the failure of WASH in other studies, pharmacologic treatment of nondiarrheal infection in children and perhaps entire households may be needed, as this may be the only way to eliminate *Cryptosporidium* from the household environment.

One limitation of our study is that we used a commercially available *Cryptosporidium* ELISA, which may be slightly less sensitive than polymerase chain reaction (PCR) or direct immunofluorescence; thus, there could be the potential for misclassification [24]. Additionally, stool specimens were collected once per week; thus, our granularity for measuring the duration of infection could be improved by biweekly or daily stool collection.

This study is unique in that it took place during the SARS-CoV-2 pandemic. Nearly one-third of participants had a positive SARS-CoV-2 IgG response. After enacting a series of nationwide lockdowns in 2020, all restrictions on movement of individuals were lifted in September 2020. Seven months later, Bangladesh experienced a record number of COVID-19 cases in April 2021, and the government imposed a strict lockdown once again. After the spike in COVID-19 cases in April 2021, we observed a corresponding spike in *Cryptosporidium* cases in May 2021 [19]. We propose the corresponding spike in *Cryptosporidium* cases could be attributed to the nationwide lockdown, as all family members were required to remain within their homes. Therefore, while social distancing was enacted to protect against the spread of SARS-CoV-2, it may have inadvertently reduced social distancing in the home, allowing for increased *Cryptosporidium* transmission among household contacts. One limitation is that the SARS-CoV-2 data are at a country-wide level. If we had accurate rates of SARS-CoV-2 infection in the Mirpur region, it would have been possible to link social-distancing measures for SARS-CoV-2 to *Cryptosporidium* cases at a community level.

This is the largest study to follow entire households over 8 months to describe the incidence of cryptosporidiosis in an endemic region. Notably, we found that prolonged shedding of cryptosporidiosis occurs among infants and adults, increasing the risk for exposure. Determining methods for interrupting transmission within the household should be prioritized to protect infants from cryptosporidiosis, especially given the lack of vaccine and approved pharmacologic treatments in this vulnerable population.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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