Species of Cryptosporidia Causing Subclinical Infection Associated With Growth Faltering in Rural and Urban Bangladesh: A Birth Cohort Study

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Background. Cryptosporidiosis is a major cause of childhood diarrhea in low- and middle-income countries and has been linked to impairment of child growth. This study investigated the burden of cryptosporidiosis and its impact on child growth in both a rural and an urban site in Bangladesh.

Methods. Pregnant women in the second trimester were identified at 2 sites in Bangladesh, 1 urban and 1 rural. Their offspring were enrolled at birth into the study (urban, n = 250; rural, n = 258). For 2 years, the children were actively monitored for diarrhea and anthropometric measurements were obtained every 3 months. Stool samples were collected monthly and during diarrheal episodes with Cryptosporidium infection and causative species determined by quantitative polymerase chain reaction assays.

Results. Cryptosporidium infections were common at both sites and mostly subclinical. In the urban site, 161 (64%) children were infected and 65 (26%) had ≥2 infections. In the rural site, 114 (44%) were infected and 24 (9%) had multiple infections. Adjusted for potential confounders, cryptosporidiosis was associated with a significantly greater drop in the length-for-age z score (LAZ) at 24 months from LAZ at enrollment (Δ-LAZ), an effect greatest in the children with multiple episodes of cryptosporidiosis. The most common species in Mirpur was Cryptosporidium hominis, whereas Cryptosporidium meleagridis predominated in Mirzapur.

Conclusions. Cryptosporidiosis is common in early childhood and associated with early growth faltering in Bangladeshi children. Predominant Cryptosporidium species differed between the 2 sites, suggesting different exposures or modes of transmission but similar consequences for child growth.

Clinical Trials Registration. NCT02764918.

Keywords. cryptosporidiosis; species; growth faltering; birth cohort; Bangladesh.

Diarrhea remains a leading global cause of morbidity and mortality in children <5 years of age [1]. Two recent multicenter studies demonstrated the importance of cryptosporidiosis, caused by an intracellular protozoan parasite, as a leading cause of childhood diarrheal disease [2, 3]. Many species of Cryptosporidium can infect humans; however, Cryptosporidium hominis and Cryptosporidium parvum are typically considered the most common [4–6]. Clinical manifestations of cryptosporidiosis are variable as symptomatic diarrheal disease occurs in only a subset of cases while most remain subclinical [7–9]. Early cryptosporidiosis, whether symptomatic or subclinical, has been associated with impaired growth and cognitive development [7, 10, 11].

Current understanding of human cryptosporidiosis is largely based upon diagnosis with overt diarrhea; yet given the associated long-term sequelae of even subclinical cryptosporidiosis, a better epidemiologic understanding in various settings is needed. In this active surveillance study, we describe the natural history of cryptosporidiosis over the first 2 years of life in 2 sites in Bangladesh, 1 urban and 1 rural. We characterize the burden of cryptosporidiosis and, using regression modeling, estimate the impact of Cryptosporidium infection on growth faltering. Furthermore, we describe a marked and unexpected difference in causative species, with C. hominis most common in the urban site compared with Cryptosporidium meleagridis in the rural site.

METHODS

Study Sites, Enrollment, and Surveillance

Prospective longitudinal birth cohorts (“Cryptosporidiosis and Enteropathogens in Bangladesh”; ClinicalTrials.gov identifier NCT02764918) were established at 2 sites in Bangladesh. Mirpur, Dhaka is a relatively poor, urban neighborhood [12,
the child was referred to the study clinic. If the SMO deter-
ness, not only diarrhea. If acutely ill during an in-home visit,
study clinic whenever the child developed symptoms of any ill-
monthly. Caregivers were encouraged to bring the child to the
child morbidity and diarrhea. The SMO assessed all children
field research assistants performed twice-weekly, in-home vis-
its to interview caregivers and collect information regarding
at Mirpur, the monthly maximum enrollment was 27.

The SMO collected demographic and socioeconomic data
using a structured questionnaire at enrollment. Thereafter,
field research assistants performed twice-weekly, in-home vis-
its to interview caregivers and collect information regarding
child morbidity and diarrhea. The SMO assessed all children
monthly. Caregivers were encouraged to bring the child to the
study clinic whenever the child developed symptoms of any ill-
ness, not only diarrhea. If acutely ill during an in-home visit,
the child was referred to the study clinic. If the SMO deter-
mined that further treatment was needed, care was provided
free of charge at the International Centre for Diarrhoeal Disease
Research, Bangladesh (icddr,b) Mirpur Treatment Centre, the
icddr,b Dhaka Hospital in Mirpur, or the Kumudini Hospital
in Mirzapur.

Height and weight were measured for mothers at the infant
enrollment visit. Infant length (to nearest 0.1 cm using length
board and plastic tape) and weight (kilograms, measured
with electronic scale; TANITA, HD-314) were obtained every
3 months. Weight-for-age z score (WAZ) and length-for-age z
score (LAZ) were determined using World Health Organization
Anthro software (version 3.2.2). The change in LAZ (Δ-LAZ)
was calculated by subtracting the enrollment LAZ from that at
24 months.

Detection of Cryptosporidiosis
Single, fresh stool specimens were collected from children every
month (monthly surveillance) and during episodes of diarrhea.
A modified Qiagen stool DNA extraction protocol was per-
formed incorporating a 95°C incubation and a 3-minute glass
bead–beating step (Qiagen, Valencia, California). All stool
samples were tested for Cryptosporidium by quantitative poly-
merase chain reaction (qPCR) assay modified from Liu et al
[15] (Supplementary Methods). A cycle threshold of 40 was
used. The pan-Cryptosporidium primers and probes target the
18S gene in multiple species known to infect humans. All sam-
ple positives for Cryptosporidium were subsequently assessed
for species identification using previously described Lib13
qPCR for C. hominis and C. parvum and a novel C. meleagridis
qPCR assay [16, 17] (Supplementary Methods).

Definitions
Diarrhea was defined as ≥3 loose stools within a 24-hour
period as reported by the child's caregiver. Infection with
Cryptosporidium was defined as detection of Cryptosporidium
DNA by qPCR from stool. Samples were grouped into a sin-
gle infection if occurring within 65 days of a preceding posi-
tive sample based upon gp60 genotyping of a subset of samples
(≤65 days: 93% concordance; >65 days: 33% concordance) [18].
Cryptosporidium infection phenotype (diarrheal or subclinical)
was based upon symptoms at the time of initial Cryptosporidium-
positive stool sample (diarrheal stool vs monthly surveillance).
Causative species was assigned based upon results of any stool
sample obtained during an infection.

Statistical Analysis
Analyses were performed using R version 3.3.3, 32-bit. The P
values for Table 1 were calculated using Wilcoxon or χ² tests.
Differences in cumulative incidence of first Cryptosporidium
infection, depicted by Kaplan-Meier curves, were assessed by
log-rank test. Association of cryptosporidiosis during the first
24 months of life with Δ-LAZ was assessed using t test initially,
and subsequently evaluated using stepwise linear regression to
adjust for potentially important confounders. Variables consid-
ered in the regression with entry P value <.1: Cryptosporidium
infection, enrollment LAZ, maternal body mass index (BMI),
household income, water source, water treatment, and exclusive
breastfeeding days. Association of recurrent Cryptosporidium
infections with Δ-LAZ was evaluated among children with 0, 1,
and ≥2 detected infections. Differences in Δ-LAZ among these
groups were tested using 1-way analysis of variance (ANOVA)
with post hoc analysis by Tukey correction for multiple com-
parisons, and further evaluated using stepwise linear regression
adjusting for the same potential confounders stated above.

Ethical Considerations
The Ethics and Research Review Committee at icddr,b approved
this study; the Institutional Review Board of the University of
Virginia provided a reliance agreement. Informed written con-
sent was obtained from a parent or guardian of each child.

RESULTS
Cohort Characterization
Pregnant women in urban Mirpur (n = 407) and rural Mirzapur
(n = 330) underwent clinical assessment, urinalysis, and ultra-
sonography in their second trimester (Figure 1). Eighteen
women at Mirpur and 54 at Mirzapur were ineligible based on
predefined exclusion criteria. Following delivery, 250 infants
at Mirpur and 262 at Mirzapur were enrolled. Reasons for
### Table 1. Comparison of Demographics, Birth Anthropometry, and Socioeconomic Indicators of Enrolled Children

| Characteristic                        | Mipur (Urban) | Mirzapur (Rural) |
|---------------------------------------|---------------|------------------|
|                                       | No Crypto (n = 89)b | Any Crypto (n = 161)b | Diarrheal Crypto (n = 51)c | Subclinical Crypto (n = 110)b | No Crypto (n = 144)b | Any Crypto (n = 114)b | Diarrheal Crypto (n = 3)c | Subclinical Crypto (n = 111)b |
|                                       | 90 (56)       | 92 (57)         | 29 (57)       | 63 (67)       | 65 (49)       | 52 (46)       | 2 (67)       | 52 (46)       |
| Female sex                            |               |                 |               |               |               |               |               |               |
|                                       | 50 (56)       | 92 (57)         | 29 (57)       | 63 (67)       | 5 (4)         | 3 (3)         |               |               |
| Household income, BDT, median (IQR)  | 140 000 (10000–21500) | 12 000 (10000–19500) | 12 000 (10000–18 000) | 12 000 (8000–20 000) | 15 000 (10 000–28 750) | 15 000 (10 000–25 250) | 35 000 (12 000–37 000) | 15 000 (10 000–25 000) |
|                                       |               |                 |               |               |               |               |               |               |
| No maternal education                  | 14 (16)       | 38 (24)         | 11 (22)       | 27 (25)       | 5 (4)         | 3 (3)         |               |               |
| Mean maternal BMI, kg/m² (SD)         | 22.8 (3.5)    | 23.0 (3.7)      | 23.0 (3.3)    | 23.0 (4.0)    | 23.3 (3.5)    | 23.1 (3.3)    | 23.2 (1.8)    | 23.1 (3.3)    |
| Mean maternal age, y (SD)             | 23.7 (4.2)    | 24.2 (4.5)      | 24.6 (4.8)    | 24.0 (4.3)    | 23.6 (4.6)    | 24.1 (4.8)    | 23.0 (3.6)    | 24.1 (4.8)    |
| Household size >5                     | 38 (43)       | 58 (36)         | 22 (43)       | 36 (34)       | 62 (46)       | 58 (51)       | 1 (33)        | 57 (51)       |
| Mean gestational age at birth, wk (SD)| 38.0 (1.7)    | 38.0 (1.8)      | 38.0 (1.8)    | 38.0 (1.9)    | 37.6 (1.7)    | 37.5 (1.8)    | 38.0 (1.0)    | 37.5 (1.8)    |
| Mean WAZ at birth (SD)                | –1.297 (0.851) | –1.287 (0.949) | –1.388 (0.927) | –1.249 (0.961) | –1.395 (0.932) | –1.339 (1.044) | –1.000 (0.573) | –1.348 (1.053) |
| WAZ > –1                              | 34 (38)       | 73 (49)         | 22 (43)       | 51 (46)       | 46 (32)       | 44 (39)       | 2 (67)        | 42 (38)       |
| WAZ = –1 to –2                        | 35 (39)       | 46 (29)         | 14 (28)       | 32 (23)       | 66 (46)       | 40 (39)       | 1 (33)        | 39 (39)       |
| WAZ = –2 to –3                        | 18 (20)       | 34 (21)         | 14 (28)       | 20 (18)       | 24 (17)       | 20 (18)       |               | 20 (18)       |
| WAZ < –3                              | 2 (2)         | 9 (6)           | 1 (2)         | 7 (8)         | 9 (8)         | 9 (8)         | 0 (0)         | 0 (0)         |
| Mean LAZ at birth (SD)                | –0.379 (0.965) | –0.386 (0.906) | –0.838 (1.029) | –0.895 (0.845) | –0.828 (1.033) | –0.858 (1.189) | –0.597 (0.621) | –0.8055 (1.201) |
| LAZ > –1                              | 43 (48)       | 83 (52)         | 28 (55)       | 55 (50)       | 66 (58)       | 2 (67)        | 2 (67)        | 64 (58)       |
| LAZ = –1 to –2                        | 31 (35)       | 59 (37)         | 20 (39)       | 33 (36)       | 37 (28)       | 32 (28)       | 1 (33)        | 31 (28)       |
| LAZ = –2 to –3                        | 15 (17)       | 17 (11)         | 2 (4)         | 15 (14)       | 20 (14)       | 10 (9)        | 0 (0)         | 10 (9)        |
| LAZ < –3                              | 0 (0)         | 2 (1)           | 1 (2)         | 1 (1)         | 2 (1)         | 6 (5)         | 0 (0)         | 6 (5)         |
| Mean exclusive breastfeeding, d (SD)  | 118 (68)      | 113 (70)        | 110 (66)      | 115 (72)      | 70 (64)       | 58 (83)       | 128 (20)      | 56 (83)       |
| Water source                          |               |                 |               |               |               |               |               |               |
| Municipal supply                      | 89 (100)      | 160 (90)        | 51 (100)      | 109 (99)      | 0 (0)         | 0 (0)         | 0 (0)         | 0 (0)         |
| Tube well                             | 0 (0)         | 0 (0)           | 0 (0)         | 0 (0)         | 135 (94)      | 111 (97)      | 3 (100)       | 108 (97)      |
| Treated water                         | 74 (83)       | 114 (71)        | 33 (65)       | 81 (74)       | 30 (21)       | 21 (18)       | 1 (33)        | 20 (18)       |
| Open drain near home                  | 32 (36)       | 60 (37)         | 21 (41)       | 39 (36)       | 7 (6)         | 1 (1)         | 0 (0)         | 1 (1)         |

Data are presented as No. (%), unless otherwise indicated.

Abbreviations: BDT, Bangladeshi taka; BMI, body mass index; Crypto, cryptosporidiosis; IQR, interquartile range; LAZ, length-for-age z score; SD, standard deviation; WAZ, weight-for-age z score.

*Children having at least 1 Cryptosporidium infection (either diarrheal or subclinical) during the first 24 months of life were included in the "Any Crypto" group. All others were included in the "No Crypto" group.

*No significant differences when compared with "No Crypto" group from Mipur by χ² or Wilcoxon test unless otherwise indicated.

*Children having at least 1 Cryptosporidium infection were further divided into "Diarrheal Crypto" or "Subclinical Crypto" based upon phenotype of initial stool sample of infection (diarrheal sample or monthly surveillance).

*No significant differences when compared with "No Crypto" group from Mirzapur by χ² or Wilcoxon test unless otherwise indicated.

*1000 BDT is equivalent to approximately 12 US dollars.

1*P = .044.
2*P = .023.
nonenrollment included parental or guardian refusal to consent, delivery after attainment of monthly or overall enrollment quota, enrollment assessment occurring >7 days after delivery, and intrauterine or neonatal death. After enrollment, 19 children at Mirpur and 8 at Mirzapur discontinued the study, primarily due to parental/caregiver withdrawal or migration. Therefore, 231 children from Mirpur and 254 from Mirzapur were actively followed. Through 30 June 2017, 212 and 254 children had completed 24 months of follow-up from Mirpur and Mirzapur, respectively. Data from children not yet completing 24 months of follow-up were included in all analyses except that of Δ-LAZ. Characteristics of enrolled children are summarized.
in Table 1 and stratified by absence or presence of at least 1 detected Cryptosporidium infection (either diarrheal or subclinical) during the surveillance period.

**Cryptosporidiosis Burden**

From 1 July 2014 through 30 June 2017, 6637 and 6417 stool samples were collected in Mirpur and Mirzapur, respectively, and tested for Cryptosporidium by qPCR (Figure 1). Fewer diarrheal stools were collected in Mirzapur (n = 271) than Mirpur (n = 1243) despite high rates of stool collection during diarrheal episodes in both sites (99% and 85% in Mirzapur and Mirpur, respectively). In Mirpur, Cryptosporidium was detected in 496 separate stool samples representing 240 distinct infections among 161 children. Of these infections, 182 were subclinical (76%, initial detection in a monthly surveillance stool) and 58 were diarrheal (initial detection in a diarrheal stool sample). Of 250 children enrolled at Mirpur, 36% (n = 89) had no detectable Cryptosporidium infections, 38% (n = 96) had 1, and 26% (n = 65) had ≥2. In Mirzapur, Cryptosporidium was detected in 186 separate stool samples representing 138 distinct infections among 114 children. One hundred thirty-five infections were phenotypically subclinical (98%) and 3 were diarrheal. Of 258 children enrolled children. One hundred infections were diarrheal or subclinical, 1351

Δ-LAZ was calculated as the difference between LAZ at 24 months and that at enrollment. Including all participants at both sites, mean Δ-LAZ for children with no detected Cryptosporidium infections was –0.386 (n = 205) vs –0.656 (n = 259) in children with at least 1 infection (P = .0062; Figure 3A). Adjusting for confounding variables (enrollment LAZ, maternal BMI, household size, household income, water source, water treatment, and exclusive breastfeeding days), cryptosporidiosis during the first 2 years of life was significantly associated with an absolute decline in Δ-LAZ of 0.215 (P = .0088; Table 2). Site-specific analysis demonstrated that cryptosporidiosis was associated with a statistically significant decrease in adjusted Δ-LAZ at Mirzapur (–0.253, P = .011), but not at Mirpur (–0.213, P = .13).

Mean Δ-LAZ was significantly different by ANOVA among children with 0, 1, or ≥2 Cryptosporidium infections (P = .0095). Post hoc analysis demonstrated that, compared with those with no episodes of cryptosporidiosis (n = 205, –0.386), mean Δ-LAZ trended lower for children with 1 episode of cryptosporidiosis (n = 174, –0.593, P = .14) and reached statistical significance for those with ≥2 episodes (n = 85, –0.786, P = .0098; Figure 3B). After covariate adjustment, children with ≥2 episodes of cryptosporidiosis had a greater decrease in Δ-LAZ (–0.2385, P = .039) than those with 1 episode (–0.2056, P = .020; Supplementary Table 3).

**Cryptosporidium Species**

Of 682 stools positive by pan-Cryptosporidium qPCR, 676 (representing 375 infections) were available for qPCR species testing. Species could not be determined for 107 infections (28%), likely due to lower sensitivity of the species-specific assay (Supplementary Methods; Supplementary Figure 1). Cryptosporidium species was determined for 166 and 102 infections for Mirpur and Mirzapur, respectively. Overall, C. hominis (total n = 148; 35 diarrheal) and C. meleagridis (total n = 100; 4 diarrheal) monoinfections were most common. Multispecies coinfections were infrequent, with 13 (5 diarrheal) C. hominis/C. meleagridis, 1 (diarrheal) C. hominis/C. parvum, and 1 (subclinical) C. parvum/C. meleagridis coinfections.

A marked difference in predominant Cryptosporidium species was observed between study sites. In urban Mirpur, C. hominis was identified in 93% of infections in which species could be determined (n = 155, 141 monoinfections; Figure 4A). Forty-one infections in which C. hominis was detected were diarrheal and 114 were subclinical. Cryptosporidium meleagridis
was the second most frequently identified species occurring in 13% (n = 22, 9 monoinfections), and *C. parvum* was identified in 2% (n = 3, 2 monoinfections). In rural Mirzapur, the predominant species was *C. meleagridis* (90%; n = 92, 91 monoinfections; Figure 4B), while *C. hominis* was identified in 7% (n = 7, all monoinfections) and *C. parvum* in 4% (total

Figure 2. Cryptosporidiosis cumulative incidence curves by study site using Kaplan-Meier method for any (A), diarrheal (B), and subclinical (C) Cryptosporidium infections. Blue line = Mirpur; red line = Mirzapur.
n = 4; 3 monoinfections). Monoinfections with C. meleagridis were nearly all asymptomatic (96%; subclinical, n = 96; diarrheal, n = 4). Of 9 diarrheal infections in which C. meleagridis was identified, 5 were coinfections with C. hominis.

DISCUSSION

We established a longitudinal birth cohort in Bangladesh comprised of 1 rural and 1 urban location to better understand the natural history of cryptosporidiosis in disparate settings. Intensive, active surveillance revealed a high burden of cryptosporidiosis with 64% and 44% of children at the urban and rural site, respectively, experiencing at least 1 Cryptosporidium infection by age 2 years. Consistent with similar prior active surveillance cohort studies in diverse geographic locations, most Cryptosporidium infections occurred in the absence of diarrhea, which we term subclinical [2, 3, 7, 9, 19]. The predominance of subclinical infection was particularly striking in rural Mirzapur where only 2% of cryptosporidiosis episodes were diarrheal. Furthermore, a marked difference between the 2 sites in the predominant Cryptosporidium species was observed, with C. hominis most common in the urban site and C. meleagridis in the rural site. Despite these differences, when the cohort was analyzed as a whole, any cryptosporidiosis during the first 2 years of life was associated with a significant decrement in growth attainment, consistent with prior studies [7, 10, 11]. This adverse association was greatest in children with ≥2 episodes of cryptosporidiosis. When considered separately, the magnitude of effect on Δ-LAZ was similar at both sites, though reaching statistical significance only in the rural site; this finding is particularly striking as nearly all Cryptosporidium infections were subclinical in this location. Collectively, these results suggest that, even without overt diarrhea, cryptosporidiosis is associated with subsequent impaired growth.

The difference in predominant Cryptosporidium species observed between the sites is striking and suggests setting-specific modes of exposure. Our finding that C. meleagridis predominated in Mirzapur contrasts with prior work showing C. hominis as the most common species in this rural site; however, that study evaluated stool collected during moderate to severe diarrhea [20]. Cryptosporidium meleagridis as a cause of medically attended diarrhea has been described in diverse geographic regions including Peru, China, and Cambodia, where it comprised 23% of Cryptosporidium-positive stools [21–24]. To our knowledge, our data from rural Bangladesh is the first description of C. meleagridis as a major species infecting children in the absence of diarrhea, yet potentially associated with a shortfall in child growth. This suggests a heretofore unrecognized burden of subclinical cryptosporidiosis attributable to a species previously considered an uncommon human pathogen. This finding has significant implications for interventions aimed at reducing Cryptosporidium-attributable morbidity. Vaccines

| Parameter                          | Parameter Estimate | P Value |
|------------------------------------|--------------------|---------|
| Cryptosporidiosis                  | -0.215             | .0088   |
| LAZ at enrollment                  | -0.606             | <.0001  |
| Maternal BMI                       | 0.035              | .0016   |
| Household size                     | -0.020             | .2833   |
| Household income (per 1000 BDT)    | 0.0065             | .0156   |
| Municipal source of water          | -0.220             | .4147   |
| Tube well source of water          | -0.043             | .8714   |
| Water source for feeding           | 0.321              | .2826   |
| Treatment of water                 | 0.146              | .1222   |
| Exclusive breastfeeding days        | -0.0015            | .0109   |

Abbreviations: BDT, Bangladeshi taka; BMI, body mass index; LAZ, length-for-age z score.
targeting C. hominis may reduce overt diarrheal cryptosporidiosis yet be ineffective in prevention of Cryptosporidium-associated developmental faltering if subclinical disease due to other species persists.

A limitation inherent to the observational design is an inability to fully account for all potentially confounding variables. Notably, testing for symptomatic or subclinical presence of enteric pathogens other than Cryptosporidium was not performed. We cannot determine whether and to what extent enteropathogens other than Cryptosporidium are contributing to growth impairment in this cohort, nor whether coinfections may result in additive or synergistic effect. It is possible that detection of Cryptosporidium is simply a surrogate indicator of some other exposure and therefore associated with, but not directly causative of, the observed growth impairment. This alternative explanation could be supported by the observation that water treatment was less common in the urban site among children who experienced cryptosporidiosis. Though statistically significant, the magnitude of difference in water treatment (83% vs 71%) seems insufficient to account for the observed differences in growth. Moreover, in the rural site where the association between cryptosporidiosis and impaired growth attainment was greatest, water treatment was infrequent, with no differences observed between children with or without cryptosporidiosis.

Figure 4. Cryptosporidium species detected and infection phenotype at urban Mirpur (A) and rural Mirzapur (B) sites. Solid bar indicates the number of diarrheal infections and open bar indicates subclinical infections.
Additional study-specific limitations include the definitions used to delimit infections and the characterization of each infection as diarrheal or subclinical. Cryptosporidium infections may be prolonged with oocysts shed intermittently and often in the absence of diarrheal symptoms [25, 26]. This, coupled with practical limitation in frequency of surveillance stool collection and use of species-level identification, precluded exact determination of when 1 infection ceased and another began. We chose to define distinct infections when >65 days separated stools with detectable Cryptosporidium based upon concordance of gp60 genotypes performed on a subset of samples. This definition could introduce bias in either direction by over- or undercounting the number of distinct infections. However, most distinct infections using this definition were separated by many months; therefore, the potential is greater for misclassifying multiple infections as a single infection, thereby underestimating the true frequency of cryptosporidiosis. Infections were classified as diarrheal or subclinical based on presence or absence of symptoms at the time of first Cryptosporidium detection. Sole use of the first timepoint of Cryptosporidium detection may bias toward overclassification of infections as subclinical, thereby underestimating somewhat the burden of diarrheal cryptosporidiosis.

Based upon our findings, we again suggest that not only diarrhea but also child growth may be considered an important clinical outcome of cryptosporidiosis. Future studies should aim to further characterize the ecology and prevalence of Cryptosporidium species, recognizing that some may infect without overt diarrhea yet still may impair growth. Further characterization of modes of transmission, which may differ by local environment and predominant species, must inform strategies to interrupt transmission. Our findings also support renewed efforts to better understand human immune responses to Cryptosporidium and elucidation of immunologic correlates of protection. It is only through such concerted and multidisciplinary efforts that cryptosporidiosis and its associated long-lasting sequelae may be abated.

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 copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Author contributions. W. A. P., R. H., and A. S. G. F. conceived the study. Field work, data gathering, and laboratory studies at icddr,b were performed by S. A., E. A., M. K., and F. T., with T. A., R. H., and A. S. G. F. providing supervision. C. B. and C. A. G. performed the species polymerase chain reaction. K. L. S., H. C., and J. Z. M. performed statistical analyses. K. L. S. wrote the manuscript with input from C. A. G., P. S. K., H. C., J. Z. M., and W. A. P. All authors contributed to revisions and approved the final version.

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