Epidemiology and Risk Factors for Cryptosporidiosis in Children From 8 Low-income Sites: Results From the MAL-ED Study

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Background. Cryptosporidium species are enteric protozoa that cause significant morbidity and mortality in children worldwide. We characterized the epidemiology of Cryptosporidium in children from 8 resource-limited sites in Africa, Asia, and South America.

Methods. Children were enrolled within 17 days of birth and followed twice weekly for 24 months. Diarrheal and monthly surveillance stool samples were tested for Cryptosporidium by enzyme-linked immunosorbent assay. Socioeconomic data were collected by survey, and anthropometry was measured monthly.

Results. Sixty-five percent (962/1486) of children had a Cryptosporidium infection and 54% (802/1486) had at least 1 Cryptosporidium-associated diarrheal episode. Cryptosporidium diarrhea was more likely to be associated with dehydration (16.5% vs 8.3%, P < .01). Rates of Cryptosporidium diarrhea were highest in the Peru (10.9%) and Pakistan (9.2%) sites. In multivariable regression analysis, overcrowding at home was a significant risk factor for infection in the Bangladesh site (odds ratio, 2.3 [95% confidence interval [CI], 1.2–4.6]). Multiple linear regression demonstrated a decreased length-for-age z score at 24 months in Cryptosporidium-positive children in the India (β = –.26 [95% CI, –.51 to –.01]) and Bangladesh (β = –.20 [95% CI, –.44 to .05]) sites.

Conclusions. This multicountry cohort study confirmed the association of Cryptosporidium infection with stunting in 2 South Asian sites, highlighting the significance of cryptosporidiosis as a risk factor for poor growth. We observed that the rate, age of onset, and number of repeat infections varied per site; future interventions should be targeted per region to maximize success.

Keywords. Cryptosporidium species; diarrhea; malnutrition; stunting; MAL-ED.

Diarrheal disease is a leading cause of death in children worldwide [1]. Cryptosporidiosis is a primary cause of moderate-to-severe diarrhea, and recent estimates suggest that annually Cryptosporidium species are responsible for >200,000 deaths in children <2 years of age in South Asia and sub-Saharan Africa, and associated with morbidity in >7 million children in these regions [2, 3]. Despite its significant impact on early childhood morbidity and mortality, cryptosporidiosis remains without a vaccine, effective treatment, or environmental intervention.

Cryptosporidium species are enteric diarrheagenic protozoa that can cause fulminant infection in immunocompromised patients and children. Cryptosporidium infection has been associated with longer duration of diarrhea and 2–3 times higher mortality in children compared with age-matched children without diarrhea [3–5]. In addition to higher mortality, studies from Brazil and Peru have noted short-term growth faltering and impaired cognitive development after Cryptosporidium diarrhea [6–8]. Beyond diarrheal disease, subclinical carriage of the parasite has been associated with growth faltering [7, 9]. The relationship between malnutrition and Cryptosporidium infection is circuitous, as stunting has been identified as a risk factor and consequence of infection [10]. Other described risk factors for Cryptosporidium infection in children include poverty [9], overcrowding [11–14], contact with domesticated animals [15, 16], and exposure to human immunodeficiency virus–infected family members [17].

Previous studies have characterized the region-specific risk factors, but we lack a community-based multisite study on the epidemiology of cryptosporidiosis in young children. The Etiology, Risk
Cryptosporidium and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) identified Cryptosporidium as a common pathogen [18] and provided the opportunity to evaluate the epidemiology of cryptosporidiosis. MAL-ED followed children for the first 2 years of life across 8 sites in South America, sub-Saharan Africa, and Asia. We aimed to understand the epidemiology, risk factors, and clinical manifestations of Cryptosporidium within this longitudinal community-based study.

METHODS

Enrollment occurred between November 2009 and February 2012 at 8 sites: Dhaka, Bangladesh (BGD); Fortaleza, Brazil (BRF); Vellore, India (INV); Bhaktapur, Nepal (NEB); Loreto, Peru (PEL); Naushero Feroze, Pakistan (PKN); Venda, South Africa (SAV); and Haydom, Tanzania (TZH) [19–26]. Children were enrolled within 17 days of birth and actively surveyed through 24 months. Ethical approval was obtained from all appropriate institutional review boards. Written informed consent was obtained from the parents. Details of the study design and microbiologic methods have been published [27, 28].

Data Collection

At enrollment, household demographics were obtained by survey, and child birthdate and sex were recorded. Baseline child length and weight were measured and subsequently collected prospectively each month. Length-for-age and weight-for-age adjusted z scores (LAZ and WAZ, respectively) were calculated. Details of illness were collected during twice-weekly household visits throughout the study period [27].

Sample Collection and Testing

Active surveillance for diarrhea was performed through interview of caregivers; during diarrheal illness, a diarrheal specimen was collected when possible. Diarrhea was defined as ≥3 loose stools per day, or at least 1 loose stool with blood; a new diarrheal episode was identified as being separated from the last by >2 diarrhea-free days [27]. Surveillance (nondiarrheal) stool specimens were collected monthly through 24 months of life; beyond year 1, only stools at months 15, 18, 21, and 24 were tested for enteropathogens. Stool specimens were collected, preserved, transported to the laboratories, and processed at all the sites using harmonized protocols. Testing for Cryptosporidium species was performed by a pan-Cryptosporidium immunoassay (TechLab, Blacksburg, Virginia). Methods of assessment of other enteropathogens were assessed using published methods [28]. All protocol-collected surveillance stools and the first diarrheal stool sample collected per diarrheal episode were included in the analysis. “Symptomatic infection” was defined as a diarrheal episode testing positive for Cryptosporidium, and “subclinical infection” was defined as a surveillance stool testing positive for Cryptosporidium. A new Cryptosporidium infection was defined as detection of Cryptosporidium in a diarrheal or surveillance stool with negative testing in the 30 days prior.

Clinical and Socioeconomic Characteristics

Dehydration was categorized as “some” dehydration, with a child being thirsty, irritable, with sunken eyes, or reduced skin turgor, or “severe” dehydration including lethargy and listlessness [27]. Diarrhea severity was scored using the Global Enteric Multicenter Study (GEMS) severity score [3]. “Moderate-severe” diarrhea was associated with dehydration, dysentery, or hospitalization, and “mild” diarrhea denoted the absence of these 3 indicators.

Monthly income was converted to US dollars and log transformed. Mothers’ schooling was categorized as follows: no school, ≤5 years, and >5 years. “Overcrowding” in the home was classified as >3 people per room per household [29]. “Unimproved” drinking water was access only to surface water or unprotected well water as compared to “improved” drinking water, which included piped water, public tap, tube well, borehole, and protected well water [30]. “Unimproved” toilet was defined as having no facility, bucket toilet, or pit latrine without slab. “Improved” toilet included nonflush pit latrine with slab and flush toilet to piped sewer system, septic tank, or pit latrine [30]. “Unimproved” household flooring was composed of earth, sand, clay, mud, or dung. “Improved” flooring was made up of wood, ceramic tiles, vinyl, or concrete [31].

Inclusion Criteria

Children were included in this analysis if they had anthropometry at baseline and at month 24. To avoid misclassification bias and be certain of Cryptosporidium-negative status, we further limited the analysis to children with complete stool testing for months 2–12 and labeled as Cryptosporidium negative, if their surveillance and diarrheal stools tested negative for Cryptosporidium during this period. Children with at least 1 Cryptosporidium-positive stool result during months 2–12 were included, and labeled as Cryptosporidium positive.

Statistical Analysis

Demographic and clinical characteristics of included children were summarized based on socioeconomic factors and environmental risk factors for enteric infection.

Evaluation of symptoms and coinfections during Cryptosporidium diarrheal episodes was performed using t tests. Logistic regression was used to determine risk factors for Cryptosporidium infection, with infection categorized as a binomial response. Variables of interest, including family income, overcrowding, years of mother’s schooling, animal ownership, floor type, drinking water source, and toilet type were included if there was >5% heterogeneity per category per site. The significant heterogeneity in characteristics between sites required independent analysis of risk factors for each site. BRF was not included in the risk factor analysis because of the limited sample size and the lack of heterogeneity for most variables.
To evaluate whether preinfection nutritional status could be a risk factor for infection, the 3-month mean LAZ score preceding the time of infection was compared between infected and uninfected children using Welch’s 2-sample t test at 4 age groups: 3, 6, 9, and 12 months.

The association of Cryptosporidium (diarrheal and subclinical) infection during year 1 and growth (LAZ) at 24 months was performed using multiple linear regression. PKN was excluded from this growth analysis due to bias noted in the anthropometric results during quality control assessments. Cryptosporidium infection was categorized as a binary variable. Covariates included in the regression differed by site based on the level of heterogeneity in variables at that site (ie, BRF showed no heterogeneity in the other variables, so they could not be included). All sites included sex and baseline LAZ and the additional following variables were included per site: (BGD: income, overcrowding; INV: income, overcrowding, toilet type; NEB: income, chickens/ducks; PEL: toilet type, chickens/ducks; SAV: income, chickens/ducks, cattle; TZH: income, chickens/ducks, cattle; BRF no additional variables).

In a second analysis to evaluate linear relationship of Cryptosporidium infection in year 1 and 24-month LAZ, we applied inverse probability weighting to account for heterogeneity across sites within a single model. Covariates included were site, sex, baseline LAZ, and the 6-month measurements of toilet type, water type, overcrowding, years of mother’s schooling, and family income.

Sequence plots were used to depict the Cryptosporidium shedding in stool over the follow-up period using the seqdef option of the TraMineR R-package. Kaplan-Meier survival analysis was used to visualize the time to first Cryptosporidium infection. All analyses were performed using Stata version 13 (StataCorp, College Station, Texas) and/or R version 3.2.2 (Foundation for Statistical Computing, Vienna, Austria) software.

RESULTS

Of 2145 children enrolled in the MAL-ED study, 1659 children completed follow-up through 24 months, and of these, 1550 children had complete 12 months of stool testing available. A subset of these (n = 1486) had complete stool testing through age 2. Baseline LAZ in BRF was significantly higher than the other sites. Most households in INV, PEL, and TZH had unimproved sanitation facilities.

Incidence of Cryptosporidium Infection

During the 2-year follow-up period, 27,418 surveillance stools were collected and tested from 1659 children, and 3.9% (1069) tested positive for Cryptosporidium, with the rate of positivity across sites ranging from 2% to 7% (Figure 1). PEL (7%) and TZH (6%) had the highest rates of subclinical infection.

From these 1659 children, 7821 diarrheal stools were collected, of which 6.9% tested positive for Cryptosporidium. The incidence of diarrhea varied greatly between sites, with PEL and PKN having the highest incidence of diarrhea overall and of diarrheal episodes positive for Cryptosporidium (Figure 2). TZH, SAV, and BRF had a low incidence of diarrhea regardless of age. Within each site, the rate of diarrhea was constant over the first 2 years of life, except in PKN where diarrheal

Figure 1. Percentage of surveillance stools positive for Cryptosporidium by age and by site. The first surveillance stool collected per child per month was included. Overall percentage of surveillance stools positive per site is summarized. Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; INV, Vellore, India; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; TZH, Haydom, Tanzania.
incidence peaked before 10 months of age but remained high through 24 months.

Across MAL-ED, 65% (962/1486) of children had at least 1 Cryptosporidium infection and 54% (802/1486) had at least 1 Cryptosporidium diarrheal episode during the first 2 years of life. By site, NEB had the lowest (21%), and PEL (62%) and TZH (68%) the highest, percentage of children with cryptosporidiosis (Table 1). BRF, TZH, PKN, and PEL were the sites with

Table 1. Characteristics of Children With Complete Follow-up, by Site

| Characteristic                              | BGD | BRF | INV | NEB | PEL | PKN | SAV | TZH |
|---------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Children included per site, No.             | 203 | 84  | 195 | 210 | 234 | 190 | 191 | 190 |
| Cryptosporidium positive, %                 | 37  | 61  | 36  | 21  | 63  | 62  | 27  | 68  |
| Female sex, %                               | 49  | 51  | 53  | 48  | 45  | 50  | 47  | 52  |
| Enrollment LAZ, mean (SD)                   | −1.0 (1.1) | −0.1 (1.2) | −1.0 (1.1) | −0.65 (1.0) | −1.3 (1.0) | −1.1 (1.2) | −0.84 (1.1) | −1.0 (1.1) |
| Exclusice breastfeeding days, median (IQR)  | 155 (117–176) | 112 (64–152) | 107 (75–138) | 86 (43–131) | 84 (29–133) | 14 (8–20) | 31 (19–52) | 55 (35–79) |
| Household monthly income, USD, median (IQR) | 108 (79–144) | 348 (308–390) | 61 (44–96) | 138 (101–211) | 127 (104–170) | 127 (81–220) | 192 (116–291) | 15 (8–30) |
| Mother’s years of schooling, median (IQR)   | 5 (2–8) | 9 (7–12) | 8 (4–10) | 9 (6–10) | 8 (6–10) | 0 (0–5) | 11 (9–12) | 7 (3–7) |
| Overcrowding, %                             | 47  | 0   | 46  | 12  | 0   | 11  | 55  | 7   |
| Poor sanitation, %                           | 0   | 0   | 56  | 1   | 76  | 23  | 4   | 87  |
| Unprotected water source, %                 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 12  |
| Cattle ownership, %                          | 2   | 0   | 4   | 3   | 0   | 64  | 16  | 66  |
| Chicken or duck ownership, %                | 7   | 6   | 10  | 34  | 40  | 50  | 39  | 88  |
| Dirt floor, %                               | 5   | 1   | 8   | 54  | 74  | 73  | 12  | 92  |

Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; INV, Vellore, India; IQR, interquartile range; LAZ, length-for-age z score; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; SD, standard deviation; TZH, Haydom, Tanzania; USD, US dollars.

Figure 2. Number of diarrheal stools collected per month per site. The sites in Peru, Pakistan, and Bangladesh had the highest incidence of diarrhea, and the sites in Peru and Pakistan had the highest rates of Cryptosporidium-positive diarrheal stools. Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; INV, Vellore, India; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; TZH, Haydom, Tanzania.
fastest progression to first infection, and all had a median time to first infection before age 1 year (Table 2). Sites varied in terms of whether diarrheal or subclinical infection occurred first. For example, in PKN, time to first diarrheal infection was earlier than subclinical. Conversely, subclinical infection occurred earlier than diarrheal infection in BGD, BRF, NEB, and PEL; and in INV and TZH, both types of infection occurred around the same age. The rate of repeat infections in year 1 varied, with PKN, PEL, and TZH having greatest repeat infections, in contrast to INV and SAV, where repeat infections during year 1 were rare (Figure 3).

Clinical Characteristics
Across sites, episodes of Cryptosporidium-associated diarrhea were clinically associated with “some” dehydration in all age strata except the 6- to 12-month group, where non-Cryptosporidium diarrheal episodes were just as likely to be associated with dehydration (Table 3); notably, there were few “severe” dehydration symptoms associated with diarrhea in this study. In children <6 months of age, Cryptosporidium diarrhea was significantly associated with a higher diarrhea severity score based on the GEMS definition [32]. Cryptosporidium-positive diarrheal episodes were not associated with fever or bloody stool (data not shown).

Copathogens
Among those children with a symptomatic Cryptosporidium infection in the first 6 months of life, one-third had a co-infection with Campylobacter, which was slightly higher than among those with no Cryptosporidium (32.8% vs 24.9%, P = .06). Conversely, in months 6–12, Cryptosporidium-negative diarrheal episodes were more likely to test positive for rotavirus (7.8% vs 2%, P = .01). No co-segregation was seen between Cryptosporidium diarrhea and other diarrheagenic pathogens including enteroaggregative Escherichia coli, Shigella, and adenovirus.

Cryptosporidium Risk Factors
Table 4 summarizes the risk factor analysis per site. In both univariate and multivariate regression analysis, overcrowding was identified as a risk factor for Cryptosporidium infection (both subclinical and diarrheal), though only significant in BGD (univariate odds ratio [OR], 2.1 [95% confidence interval [CI], 1.1–3.9]; multivariate OR, 2.33 [95% CI, 1.2–4.6]) (Table 4 and Supplementary Table 1). Children with a lower preceding mean 3-month LAZ were more likely to have a Cryptosporidium infection (6-month: LAZ –1.0 vs –1.2, P = .06; 9-month: LAZ –1.3 vs –1.1, P = .05; 12-month: LAZ –1.6 vs –1.3, P = .007) (Supplementary Table 2).

Cryptosporidium Infection as Predictor of Growth
We evaluated the relationship between Cryptosporidium infection during the first year of life and its impact on LAZ at 24 months using linear regression for each of 7 sites (PGN excluded, 1328 children). In 2 South Asian sites, INV and BGD, children with a Cryptosporidium infection during year 1 had a 0.25 lower LAZ at 24 months (INV: β = –.26 [95% CI, –.51 to –.01]; BGD: β = –.25 [95% CI, –.49 to –.01]) compared to children without a Cryptosporidium infection during year 1 (Table 5), but this association was not seen in the other sites (Figure 4). A similar trend was noted in INV and BGD for LAZ at 12 and 18 months (Supplementary Table 3). Linear regression of Cryptosporidium infection on the 24-month LAZ across sites using inverse probability weighting to accommodate multiple risk factors gave similar results (β = –.08 [95% CI, –.22 to .06]).

| Table 2. Median Time to First Cryptosporidium Infection, by Site |
|---------------------------------|----------------|----------------|----------------|
| Site | Median Days (SD) | Number of Subjects | Number of Events | Median Days (SD) | Number of Subjects | Number of Events | Median Days (SD) | Number of Subjects | Number of Events |
|-----|----------------|-----------------|----------------|----------------|-----------------|----------------|----------------|-----------------|----------------|
| BGD | 381 (128) | 203 | 74 | 235 (113) | 17 | 17 | 124 (134) | 57 | 57 |
| BRF | 188 (149) | 84 | 51 | 294 (77) | 3 | 3 | 61 (81) | 48 | 48 |
| INV | ... | 195 | 71 | 272 (102) | 14 | 14 | 248 (110) | 57 | 57 |
| NEB | ... | 210 | 44 | 161 (103) | 9 | 9 | 93 (113) | 35 | 35 |
| PEL | 274 (132) | 243 | 154 | 234 (102) | 48 | 48 | 123 (112) | 106 | 106 |
| PKN | 272 (125) | 243 | 146 | 139 (93) | 69 | 69 | 212 (106) | 77 | 77 |
| SAV | ... | 190 | 51 | ... | ... | ... | 273 (94) | 51 | 51 |
| TZH | 249 (129) | 191 | 130 | 143 (105) | 8 | 8 | 153 (112) | 122 | 122 |

The first column shows median time to first Cryptosporidium infection, including both diarrheal and subclinical infections. Only data from year 1 of life were included, due to incomplete testing for Cryptosporidium year 2 surveillance stools. INV, NEB, and SAV all had median time to first infection beyond 1 year, so median times could not be calculated using this model. The second column shows median time to first diarrheal infection. The third column shows median time to first subclinical infection. For SAV, there were no Cryptosporidium diarrheal events during this time period.

Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; INV, Vellore, India; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; SD, standard deviation; TZH, Haydorn, Tanzania.
DISCUSSION

This is the first multicountry prospective cohort study of clinical and predictive risk factors for Cryptosporidium infection in community-dwelling children using harmonized protocols across 8 sites. This study provides incidence of diarrheal and subclinical Cryptosporidium infection in children from birth to 2 years of age from sites in South Asia, sub-Saharan Africa, and South America.

Our study of 1486 children demonstrated that Cryptosporidium is a common pathogen, affecting 65% of subjects, and is associated with diarrheal illness in 54%. In all sites, Cryptosporidium infection in the first 6 months of life was associated with more severe illness as measured by the GEMS severity score. The primary driver of severity was likely dehydration.

Other clinical signs, including fever, dysentery, and vomiting, were not associated with Cryptosporidium diarrhea. And although severe dehydration was rare in this community-based study, Cryptosporidium diarrheal episodes were more likely associated with some dehydration vs non-Cryptosporidium diarrheal episodes. These findings suggest that younger children are more susceptible to severe Cryptosporidium disease, and that community-based programs for oral rehydration may play an important role in limiting morbidity from Cryptosporidium, as with other diarrheal pathogens.

In addition to diarrhea-associated infections, ours is the first longitudinal study to report rates of subclinical Cryptosporidium infection in 8 sites. We found that Loreto, Peru (7%) and Haydom, Tanzania (6%) were sites with highest rates

Figure 3. Time to Nth Cryptosporidium detection over the first year of life. This includes both diarrheal and asymptomatic Cryptosporidium infection. Children at the Peru, Pakistan, and Tanzania sites frequently had up to 3 infections with Cryptosporidium. Repeat infection was less commonly observed in the other 5 sites. Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; INV, Vellore, India; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; TZH, Haydom, Tanzania.
of subclinical Cryptosporidium infection, which is significant as the burden of subclinical infections in these locations has not been previously reported at this scale. Also of note, in Fortaleza, Brazil, though children had a better nutritional status at enrollment, and less diarrhea, we found the incidence of subclinical Cryptosporidium infection to be 3%, suggesting that subclinical infection is an important problem at this site. The incidence of subclinical infections in South Asia was lower than previously reported [9, 33, 34]. This difference likely reflects differences in testing. For the current study, we used antigen detection for

| Characteristic | <6 mo | 6–11 mo | 12–17 mo | 18–24 mo |
|----------------|-------|---------|----------|---------|
| No. Vomiting, % | 16.0 | 21.8 | 18.2, 173 | 1.0 | 14.2 | 8.2 | .06 | 8.2 | 10.1 | .62 |
| Fever, % | 3.3 | 3.0 | .67 | 6.3 | 7.3 | .35 | 4.7 | 4.8 | .99 | 4.5 | 4.5 | .10 |
| Days of diarrhea (mean) | 5.7 | 6.29 | 5.98 | 5.28 | .66 | 4.82 | 5.31 | .21 | 4.31 | 3.32 | 4.92 | 3.38 | .034 | 4.00 | 3.41 | 3.73 | 2.54 | .41 |
| Dehydration, % | .005 | .22 | .047 | .002 |
| None | 91.5 | 83.5 | ... | 89.2 | 84.8 | ... | 89.5 | 84.2 | ... | 90.9 | 90.0 | ... |
| Some | 8.3 | 16.5 | ... | 10.7 | 15.2 | ... | 10.5 | 15.8 | ... | 9.1 | 10.0 | ... |
| Severe | 0.1 | 0.0 | ... | 0.0 | 0.0 | ... | 0.0 | 0.0 | ... | 0.0 | 0.0 | ... |
| Moderate-severe diarrhea (GEMS definition), % | 11.9 | 20.3 | .007 | 15.4 | 19.2 | .26 | 14.3 | 17.8 | .98 | 14.6 | 12.7 | .70 |
| Diarrhea >14 days, % | 6.7 | 8.3 | .59 | 3.2 | 4.0 | .80 | 1.9 | 2.1 | 1.0 | 1.4 | 0.9 | 1.0 |
| Presence of other pathogen, % | ... | ... | ... | ... |
| Any bacteria | 51.8 | 52.7 | .92 | 69.6 | 69.5 | 1.0 | 71.1 | 71.8 | .93 | 74.7 | 70.5 | .41 |
| Any virus | 25.0 | 28.6 | .42 | 42.0 | 37.1 | .27 | 36.7 | 29.5 | .10 | 30.7 | 23.6 | .15 |
| Adenovirus | 2.5 | 15.6 | .66 | 5.2 | 2.6 | .23 | 4.8 | 1.4 | .09 | 4.8 | 3.6 | .75 |
| Astrovirus | 4.4 | 4.5 | 1.0 | 5.8 | 4.6 | .67 | 7.2 | 4.8 | .36 | 4.8 | 3.6 | .75 |
| Rotavirus | 4.1 | 4.5 | 1.0 | 7.8 | 2.0 | .013 | 6.6 | 3.4 | .19 | 6.2 | 3.6 | .39 |
| Shigella | 0.2 | 0.8 | .41 | 1.2 | 0.7 | .87 | 4.0 | 2.8 | .61 | 7.0 | 5.5 | .71 |
| Campylobacter | 24.6 | 32.8 | .056 | 44.0 | 46.4 | .64 | 46.7 | 47.9 | .83 | 48.0 | 47.3 | .96 |
| Giardia | 6.7 | 10.5 | .13 | 15.6 | 23.2 | .019 | 24.8 | 23.3 | .77 | 35.6 | 32.7 | .62 |
| EAEC | 25.7 | 22.9 | .54 | 26.9 | 21.2 | .15 | 21.0 | 19.3 | .72 | 17.3 | 23.3 | .16 |
| 0–6 mo | 55 | 29 | ... | ... |
| 6–12 mo | 22 | ... | ... | ... |
| 12–18 mo | ... | ... | ... | ... |
| 18–24 mo | ... | ... | ... | ... |

Significant P values listed in bold.
Abbreviations: –, non-Cryptosporidium diarrheal episode; +, Cryptosporidium-associated diarrheal episode; EAEC, enteroaggregative Escherichia coli; GEMS, Global Enteric Multicenter Study.

| Risk Factor | Odds Ratio (95% CI) |
|-------------|---------------------|
| Overcrowding | 2.1 (1.1–3.9) |
| Dirt floor | 1.8 (1.6–2.7) |
| Poor sanitation | 1.5 (0.85–2.64) |
| Unprotected water source | ... |
| Chickens or ducks kept in home | 0.86 (0.1–3.1) |
| Cattle kept in home | 0.86 (0.1–3.1) |
| Maternal schooling 1–5 y | 0.86 (0.38–2.7) |
| Maternal schooling >5 y | 0.86 (0.38–2.7) |
| Household income (log) | 1.05 (0.6–1.86) |

Variables with <5% heterogeneity between subcategories in this site were not included in analysis due to lack of power (indicated by ellipses).
Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; CI, confidence interval; INV, Vellore, India; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; TZH, Haydom, Tanzania.
Cryptosporidium as compared to the use of the higher-sensitivity quantitative polymerase chain reaction in the prior Indian and Bangladeshi studies [9, 32, 33]. A recent reanalysis of the GEMS study has shown that enzyme-linked immunosorbent assay (ELISA) may be just as sensitive as molecular diagnostics for Cryptosporidium in diarrheal disease [35]. However, as non-diarrheal subclinical infection carries a lower parasite burden, the ELISA may have underestimated incidence of subclinical infection in MAL-ED, and more sensitive testing is warranted.

Overcrowding, lower 3-month growth velocity, poor sanitation, and poultry were associated with increased risk of infection. Overcrowding is a known risk factor for cryptosporidiosis [11–14] and may be a corollary for lower socioeconomic status; alternatively, overcrowding in the home may increase risk of person-to-person transmission between household members.

We did not find an association of cryptosporidiosis with unprotected drinking water, supporting results of an Indian study that demonstrated no reduced risk of cryptosporidiosis with drinking bottled water vs water from the municipal supply [36]. This suggests that other factors, including crowding, poor sanitation, and high environmental burden, may promote transmission of infection to young children.

This study demonstrated a significant association of Cryptosporidium infection during year 1 and linear growth faltering at 24 months of age in INV and a trend toward significance in BGD, consistent with a prior study from this region [9]. In the other sites, no significant relationship between infection and LAZ score at 24 months was observed. There are several potential reasons for these divergent findings. Our model did not account for nutritional intake as a component of growth, though other MAL-ED publications have evaluated this and found no significant signal [37]. In addition, the epidemiology and prevalence of different Cryptosporidium species and subspecies has not been extensively described, and may differ greatly across the sites we studied. Molecular studies have suggested differences in clinical presentation between Cryptosporidium species; for example, Cryptosporidium hominis has been associated with more severe dehydration, and Cryptosporidium meleagris with mild disease [6, 37, 38]. Thus, different species may also vary in pathogenic impact on child growth. It is also possible that there is residual confounding of unknown risk factors.

A novel finding from this study is the variability in the age of onset of Cryptosporidium infection. In BRF, PEL, PKN, and TZH the median age at first infection was in the first year of life whereas in the other 4 sites, the median age was >1 year. The finding from BGD is consistent with a prior report from Bangladesh, which reported median age at first infection to be 13.9 months [9]. In the sites with earliest age of onset, there was also the greatest rate of repeat infections. Earlier onset of infection and greater probability of repeat infection could be due to greater burden of circulating parasite in that environment. This could also be attributed to failure of host immune response, either related to host genetics or lack of protection from maternal breast milk antibodies [32, 39]. It is also possible that infection by one Cryptosporidium species does not afford protection from all other species, and in sites with repeat infections there is greater genotypic diversity of the parasite, increasing risk of recurrent infection. Further studies are needed to understand whether acquired immunity to Cryptosporidium is species or genotype specific.

This study had limitations. Our analysis was limited to including infections in year 1 of life, due to incomplete testing in year 2 per the study design, and did not account for the impact of a large burden of infections that occurred in the second year of life. Although we have details on some of these infections, we lacked complete follow-up so could not categorize someone as not having an infection, and similarly a child may have had an infection and resolved it.

Furthermore, heterogeneity in multiple factors between sites, as well as limited sample sizes within sites, significantly impaired ability to draw cross-site and per-site conclusions. Last, we were unable to test infection in household members and environmental samples to more accurately describe exposure risk.

MAL-ED represents the largest multicountry longitudinal investigation of Cryptosporidium infection in children. Our study confirmed that Cryptosporidium infection is a significant contributor to diarrhea morbidity in community-dwelling children, and found that a majority of children across sites experienced infection before age 2. The differences in age of onset, diarrhea-associated or subclinical infection, and the rate of repeat infections across sites indicates that site-specific characteristics must be considered when designing infection control strategies for Cryptosporidium. Given our findings of associations between Cryptosporidium and short- and long-term morbidity, efforts at prevention and control of the parasite should focus on areas that have seen high rates of infection in the

Table 5. Linear Regression of Association of Cryptosporidium Infection (Includes Both Diarrheal and Subclinical) in First 12 Months of Life and Length-for-Age z Score at 24 Months

| Site   | No. | β (95% CI) |
|--------|-----|------------|
| BGD    | 172 | −.20 (−.44 to .05) |
| BRF    | 70  | .00 (−.47 to .48) |
| INV    | 187 | −.26 (−.51 to −.01) |
| NEB    | 204 | .06 (−.20 to .32) |
| PEL    | 186 | −.13 (−.36 to .09) |
| SAV    | 128 | .13 (−.23 to .49) |
| TZH    | 165 | .19 (−.09 to .48) |

Total No. of children included was 1328 (222 children from Pakistan were excluded).

Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; CI, confidence interval; INV, Vellore, India; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; TZH, Haydom, Tanzania.
first year of life (PEL, PKN, TZH) and an association between Cryptosporidium infection and malnutrition (BGD and INV).

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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Figure 4. Forest plot depicting estimates of multiple linear regression of length-for-age z score at 24 months and Cryptosporidium infection during first 12 months of life, by site. Sites are ordered by burden of Cryptosporidium infection, with the Peru site having the most infections, and the Brazil site having the fewest. Abbreviations: BGD, Dhaka, Bangladesh; BRF, Fortaleza, Brazil; INV, Vellore, India; LAZ, length-for-age z score; NEB, Bhaktapur, Nepal; PEL, Loreto, Peru; PKN, Naushero Feroze, Pakistan; SAV, Venda, South Africa; TZH, Haydom, Tanzania.
Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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