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Miranda Delahoy Date

Environmental, Behavioral, and Clinical Characteristics Associated with *Cryptosporidium* Infection among Children Less than Five Years Old with Moderate-to-Severe Diarrhea in Rural Western Kenya

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Bachelor of Arts

Oberlin College

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Thesis Committee Chair: Clair Null, PhD

An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

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Abstract

Environmental, Behavioral, and Clinical Characteristics Associated with *Cryptosporidium* Infection among Children Less than Five Years Old with Moderate-to-Severe Diarrhea in Rural Western Kenya

By Miranda Delahoy

Purpose: Outbreaks of *Cryptosporidium* in developed countries have been studied in detail; however, less is known about risk factors for endemic cryptosporidiosis. The purposes of this study are: to describe the prevalence of *Cryptosporidium* infections in children less than five years old with moderate-to-severe diarrhea enrolled in the rural western Kenya site of the Global Enteric Multicenter Study (case children), to assess environmental, behavioral, and clinical characteristics associated with *Cryptosporidium* infections, and to describe the outcomes and consequences of *Cryptosporidium* infections among these children.

Methods: Stool samples were tested at enrollment for presence of enteric pathogens, data on environmental, behavioral, and clinical characteristics were collected at enrollment, and each child's health status was recorded at a 60-day follow-up. Data were analyzed using multivariable logistic regression to assess characteristics associated with *Cryptosporidium*.

Results: Overall, 10.8% of 1,476 case children had a *Cryptosporidium* infection and 4.6% of these children had died by their 60-day follow-up. There was no significant difference between the proportion of children with and without *Cryptosporidium* infections who were underweight or wasted at baseline; however, those with *Cryptosporidium* infections were significantly more likely to be underweight or wasted by the time of follow-up. Characteristics significantly positively associated with *Cryptosporidium* included age less than two years, producing a watery stool sample at enrollment, and having flaky skin at enrollment. Characteristics significantly negatively associated with *Cryptosporidium* were having a rotavirus infection, coming from a household in which rainwater was the main source of drinking water or a household that reports boiling drinking water, and coming from a household in which the caretaker reports washing hands after defecation.

Discussion: *Cryptosporidium* contributes significantly to the burden of diarrheal illness in the study site, particularly among those less than two years old. Rapid and reliable diagnostic tests for *Cryptosporidium*, improved diarrhea case management and treatment of cryptosporidiosis, vaccine research, adequate access to improved drinking water, and future research on ceramic water filters are recommended for reducing the burden of cryptosporidiosis in rural western Kenya.

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CONTENTS

INTRODUCTION

BACKGROUND

Diarrheal disease is a major cause of morbidity and mortality in developing countries[1]. The Global Enteric Multicenter Study (GEMS), a case-control study of moderate-to-severe diarrhea (MSD) in seven countries in South Asia and sub-Saharan Africa, was undertaken to assess the burden and etiology of MSD in its study sites. Population attributable fractions were calculated by GEMS investigators to assess which pathogens episodes of MSD could be attributed to. In all African sites (The Gambia, Mali, Kenya, and Mozambique) *Cryptosporidium* had the second-highest attributable fraction in the infant age category (0-11 months). In GEMS Kenya, *Cryptosporidium* was a major pathogen in all GEMS age strata (0-11 months, 12-23 months, and 24-59 months)[2].

Cryptosporidium, a protozoan parasite that has a low infectious dose and is highly tolerant to disinfection with chlorine[3], is an important cause of diarrheal illness in the developing world, especially among immunocompromised individuals[4]. A vaccine for *Cryptosporidium* is not currently available and while treatment with nitazoxanide has been shown to effectively treat cryptosporidiosis in immunocompetent patients[5], this treatment is not often used in developing countries[6] and furthermore no significant benefits of this treatment were observed when it was randomized to HIV-infected children in a study in Zambia[7]. In the United States, nitazoxanide is approved for treatment of cryptosporidiosis only for those older than one year of age and is not approved for infants[8].

Cryptosporidium infections have been associated with prolonged and persistent diarrhea[9-15]. *Cryptosporidium* infections in infants in developing countries have been associated with growth faltering[16], excess mortality[17], and an excess burden of diarrhea later in life[18].

The purposes of this study are: (1) to describe the prevalence of *Cryptosporidium* infections in children less than five years old with MSD enrolled in the GEMS rural western Kenya site, (2) to assess environmental, behavioral, and clinical characteristics associated with *Cryptosporidium* infection, including demographic and household characteristics, clinical signs and symptoms of illness, nutritional status, presence of other enteric pathogens in stool, breastfeeding practices, HIV status, drinking water source, sanitation facilities, hygiene behaviors, and temperature and rainfall, and (3) to describe the outcomes and consequences of *Cryptosporidium* infections among these children.

LITERATURE REVIEW

Cryptosporidium Characteristics and Transmission

Cryptosporidium is a protozoan parasite transmitted in drinking and recreational water, food, from person to person, and from animals to people[19]. Airborne transmission of *Cryptosporidium* has also been reported[19, 20]. *Cryptosporidium* was first recognized by Ernest Tyzzer in mice in 1907 and has been widely studied since the late 1970s and early 1980s when cryptosporidiosis was found in several patients with AIDS[21, 22]. *Cryptosporidium* species have since been identified in more than 150 mammalian species[23] as well as in birds, reptiles, fish, and amphibians[24].

Cryptosporidium oocysts are immediately infectious upon shedding, contributing to the ease of person to person transmission[21]. Oocysts are also very small (4-6µm)[23] and can survive in cool, moist environments for months[24]. The infectious dose of *Cryptosporidium* has been estimated through challenge studies[25] and outbreak models[26]. Results of these studies show that *Cryptosporidium* is highly infectious with a median infectious dose as low as nine oocysts[25]. Humans and animals with cryptosporidiosis can shed large quantities of oocysts for several weeks, also contributing to the transmission of the parasite[6, 27].

Diagnostic Techniques for *Cryptosporidium* and *Cryptosporidium* Genotypes

A histologic examination of intestinal mucosa was required to diagnose cryptosporidiosis before the 1980s[19, 27]. Less invasive tests have since become available. Modified acid-fast staining and negative staining can be used to examine *Cryptosporidium* oocysts from fecal samples[19]. Enzyme-linked immunosorbent assays (ELISAs) can also be used to identify *Cryptosporidium* and restriction fragment length polymorphism analysis (RFLP) of DNA can be used to differentiate *Cryptosporidium* species[28]. Serological testing has also been used to estimate prior exposure to *Cryptosporidium*[29]. Estimates of *Cryptosporidium* prevalence can vary based on the choice of diagnostic method[6].

Cryptosporidium species are not all host specific[24] and the taxonomy of *Cryptosporidium* species is still evolving[28]. The two most common species of *Cryptosporidium* found in humans are *C. parvum* and *C. hominis*[30] and other species identified in humans include *C. canis*, *C. meleagridis*, and *C. felis*[3, 31]. *C. hominis* was found to be associated with greater growth shortfalls compared to *C. parvum* among children in Brazil[32].

Clinical Features of *Cryptosporidium* Infection in Humans

Cryptosporidiosis is usually a self-limiting infection in immunocompetent individuals, but can cause chronic diarrhea and occasionally death in an immunocompromised human host[33]. *Cryptosporidium* can be symptomatic or asymptomatic. Symptoms are thought to begin one to four weeks after infection[34]. The most common characteristics of symptomatic infection include watery diarrhea, fever, and vomiting[35, 36]. Mucus is often present in the stools of individuals infected with *Cryptosporidium*[12, 37].

Cryptosporidium has been associated with prolonged diarrhea (lasting 7-13 days) and persistent diarrhea (lasting 14 days or longer) in children[9-15]. In a study of cryptosporidiosis in children in northeastern Brazil, the mean duration of an episode of diarrhea associated with *Cryptosporidium* was 12 days[18]. Among immunocompetent individuals in another study, the mean duration of symptoms was also 12 days[34]. Duration of diarrhea related to *Cryptosporidium* has been recorded in excess of two months[12].

Cryptosporidium infections have been recorded as at least a contributing cause of death among malnourished children[37] and *Cryptosporidium* has been associated with excess mortality in children who become infected in infancy[17] and early childhood[15].

Cryptosporidium Infections in Immunocompromised Individuals

Cryptosporidium is an opportunistic pathogen making HIV-positive individuals at high risk for cryptosporidiosis. Upon examination of stool samples from patients presenting to a health facility in Ethiopia, 43.6% of HIV-positive individuals were found to have a *Cryptosporidium* infection[38]. In a study of HIV-associated diarrhea in Nairobi, *Cryptosporidium* was the most common pathogen detected among HIV-positive patients with chronic diarrhea and mortality was significantly higher among those with a *Cryptosporidium* infection compared to those with chronic diarrhea who did not have a *Cryptosporidium* infection[39]. Diarrhea associated with *Cryptosporidium* in AIDS patients can last for months[40]. Cryptosporidiosis can be serious, with high levels of oocyst shedding, and can lead to death among those with AIDS[40, 41]. Cryptosporidiosis in AIDS patients has been associated with nutrient malabsorption and altered intestinal permeability, especially among those with a heavy burden of cryptosporidiosis[40]. Such changes to intestinal architecture can contribute to malnutrition.

Nitazoxanide is an effective treatment for cryptosporidiosis among immunocompetent individuals; however, it was not found to be beneficial to those who are HIV-seropositive[5]. Treatment with antiretrovirals has been found to improve diarrhea and improve or eliminate cryptosporidial infections in HIV-positive individuals[41, 42].

Cryptosporidium in Children

Cryptosporidium is common in children less than one year of age in developing countries[19]. Studies of *Cryptosporidium* in children often focus on children less than five years of age and several have shown that *Cryptosporidium* infections in children are most common in the younger age groups and begin to drop off after the age of two years[17, 35, 37, 43, 44]. The lower prevalence in older age groups is thought to be an effect of acquired immunity[6, 45-47]. Checkley *et al.* found that symptomatic cryptosporidiosis was more common in older age groups in children under two years old, whereas children under six months of age had more asymptomatic infections[48].

Breastfeeding has been found to be protective against *Cryptosporidium*[36]. In sub-Saharan Africa, the highest prevalence of cryptosporidiosis is often seen in the 6-12 month age group: it is thought that breastfeeding may offer protection before the age of six months[47].

Clinical symptoms of cryptosporidiosis in malnourished children include gastroenteritis, fever, vomiting, dehydration, and persistent diarrhea lasting more than 14 days[37].

Growth Faltering, Malnutrition, and Developmental Deficiencies

Both symptomatic and asymptomatic *Cryptosporidium* infections have been associated with decreased growth in children[16, 48]. Additionally, *Cryptosporidium* infections in children in developing countries have been associated with malnutrition, development delays, and decreased physical fitness abilities following infection[30, 49]. *Cryptosporidium* infections in children less than one year of age have been associated with an increased burden of subsequent diarrhea episodes which have been reported to continue for nearly two years (approximately 21 months)[18].

After infection with *Cryptosporidium,* children's height and weight increase more slowly than they do for children in their age group who did not have a *Cryptosporidium* infection[16]. The effects on growth were greater among children infected at an earlier age[16]. While there was catch-up weight gain that was usually completed after six months, children with *Cryptosporidium* often do not catch up to non-infected children in height growth[16].

Although some weight and height growth deficits among children infected with *Cryptosporidium* infection during childhood may be recovered through catch-up growth, there are long-term physical fitness deficits associated with early childhood *Cryptosporidium* infections[49]. A *Cryptosporidium* infection in the first two years of life was associated with subsequent physical functional deficits based on a Harvard Step Fitness test score for 4-7 years after the *Cryptosporidium* infection[49].

Stunting that was present before a *Cryptosporidium* infection was associated with greater effects on growth for a longer period of time[16]. Studies have shown that those who are malnourished are more susceptible to *Cryptosporidium* infection, indicating a "complex bidirectional relationship"[6] between *Cryptosporidium* and malnutrition.

Known Risk Factors for *Cryptosporidium* Infection in Developing Countries

Although outbreaks of *Cryptosporidium* in developed countries have been studied in detail, less is known about risk factors for endemic cryptosporidiosis[50]. Breastfeeding has been shown to be protective against *Cryptosporidium* infections[36]. Children whose families kept prepared food for later consumption were found to be at higher risk for *Cryptosporidium* infection in Guinea-Bissau, as were those who consumed stored food or had dogs or pigs in their houses[13].

Water Treatment for *Cryptosporidium* Removal or Inactivation

Cryptosporidium is highly tolerant of chlorine[21, 51]. Solar ultraviolet light can inactivate *Cryptosporidium* oocysts[24, 52] and solar water disinfection (SODIS) has been suggested as a cost-effective method for inactivation of *C. parvum* oocysts in water[52]. Ceramic filters can be used to remove *Cryptosporidium* oocysts from drinking water.

Treatment, Management, and Prevention of Cryptosporidiosis

Nitazoxanide has been found to alleviate symptoms and oocyst shedding from cryptosporidial infections among immunocompetent individuals; however, it has not shown to be effective among those who are HIV-seropositive[5] and is not indicated for infants[8]. A review found that paromomycin and azithromycin have been found to be effective against *Cryptosporidium* in immunocompetent adults but either did not show a response among infants or were not tested among infants and children[19]. The WHO recommends oral rehydration therapy and zinc supplementaion for uncomplicated diarrhea management (in absence of severe dehydration)[53].

There is currently no vaccine available for *Cryptosporidium*; however, the evidence of acquired immunity suggests that a vaccine could be effective[6]. Improved water, sanitation, and hygiene, as well as promotion of exclusive breastfeeding of infants may also be effective at preventing cryptosporidiosis.

Seasonality of *Cryptosporidium* Infections in Developing Countries

Cryptosporidium detection shows a seasonal pattern in a number of countries[12, 15, 17, 35, 44, 54-60]. In India, Kenya, Guinea Bissau, Uganda, Malawi, Zambia, Guatemala, Brazil, and Mexico seasonality of *Cryptosporidium* appears to be related to rainfall[12, 15, 17, 35, 44, 55-57, 59, 60]. In northeast India, Mexico, Brazil, Uganda, Malawi, and Guinea Bissau *Cryptosporidium* infections peaked during the rainy season[15, 17, 35, 55, 59, 60]; however, in Delhi, India, *Cryptosporidium* infections in children peaked in hot, dry months[57]. In a retrospective study of *Cryptosporidium* infections in South Africa, the majority of *Cryptosporidium* cases also presented in the hot, dry months[58]. A study in Guatemala found most cases of *Cryptosporidium* to be at the end of the dry season[12]. In a study of *Cryptosporidium* during the rainy season in Zambia, infections peaked early in the rainy season and were much lower later in the season[56]. In one study in Kenya, human infections were highest in the dry season[44] and oocyst detection in surface water was highest at the end of the rainy season[61].

Hypotheses relating to a rainfall-driven seasonality of *Cryptosporidium* prevalence include rain flushing *Cryptosporidium* oocysts into surface water[55, 61], improved survival of oocysts in wet conditions[19, 47], dusty conditions leading to respiratory *Cryptosporidium* infections in the dry season[12], poor hygiene during water shortages[12, 44], children playing in surface water in rainy seasons[55], increased immunity after the beginning of a rainy season[47], and use of alternative water sources in the dry[44, 47] or rainy[55] season.

Global Burden of Disease from *Cryptosporidium*

The lack of a readily-available rapid test for *Cryptosporidium* makes it difficult to assess the global burden of disease from cryptosporidiosis[6]. *Cryptosporidium* has been found in more than 90 countries worldwide and on all continents except Antarctica. In a review, it was summarized that *Cryptosporidium* oocysts have been detected in 2.6-21.3% of fecal samples in African countries, 3.2-31.5% of samples from Central and South American countries, in 0.1-14.1% of fecal samples from Europe, in 0.3-4.3% of samples from North, America, and in 1.3-13.1% of countries in Asia, the Pacific, and Caribbean areas[24].

***Cryptosporidium* in Kenya**

Few studies have examined human cryptosporidiosis in Kenya. Gatei *et al.* analyzed fecal samples from children less than five years old that were submitted for ova and parasite inspection at six microbiology laboratories in Kenya for detection of *Cryptosporidium*[44]; previously tests for *Cryptosporidium* were not routinely performed[44, 62]. The prevalence of *Cryptosporidium* was 4% in the study and was highest in what is typically the dry seasons in Kenya[44]. Prevalence of *Cryptosporidium* was highest among those ages 13-24 months and lowest among those who were 49-60 months[44]. *Cryptosporidium* infection was significantly associated with persistent diarrhea lasting more than 14 days, vomiting, and abdominal swelling[44]. Of the samples for which genotype information was available, 87% of isolates in the study were of the genotype *C. hominis*[44].

In a survey of fecal parasites, parasites were present in 30 of 100 fecal samples from patients with diarrhea and abdominal pain presenting at two hospitals in western Kenya[62]. In this survey *C. parvum* was the most commonly detected parasite and was found in five patients[62].

A study examining chronic diarrhea in HIV-positive adults in Nairobi found *Cryptosporidium* to be the most common pathogen in this population and that *Cryptosporidium* was significantly associated with mortality among HIV-infected adult patients with chronic diarrhea[39].

Muchiri *et al.* tested surface water in Meru, Kenya for *Cryptosporidium* oocysts and found a peak in oocyst detection at the end of the rainy season[61].

Other studies of *Cryptosporidium* in Kenya focused on highly specific populations such as urban dairy farmers in Dagoretti, Nairobi[63] or pathogen etiology among Maasai children[64].

METHODS

Global Enteric Multicenter Study (GEMS): Overview and Enrollment

Data for the study was collected as part of the Global Enteric Multicenter Study (GEMS), a case-control study of children less than five years old with moderate-to-severe diarrhea (MSD) in seven countries in South Asia and sub-Saharan Africa carried out from 2008 to 2011. The rationale, study design, clinical and microbiologic methods, and assumptions of GEMS have recently been described elsewhere[65-68]. Briefly, GEMS aimed to enroll 220 MSD cases per year from selected sentinel health facilities serving each site in each of three age strata (0-11, 12-23, and 24-59 months old), along with 1-3 matched community controls. Cases and controls supplied clinical, epidemiologic, and anthropometric data at enrollment and again 60 days later, and provided enrollment stool specimens for identification and characterization of potential diarrheal pathogens. This paper focuses specifically on the data collected at the rural western Kenya site and the analysis is restricted to case children presenting to sentinel health facilities with MSD.

Inclusion criteria for case children with MSD: a GEMS case child was considered eligible for enrollment if they met the following criteria: was less than five years old; resided in the local demographic surveillance system catchment area; presented at a sentinel health facility within seven days of illness onset with MSD. MSD was defined as having three or more loose stools in the previous 24 hours, without diarrhea in the seven days prior, and having one or more of the following illness severity characteristics: loss of skin turgor, sunken eyes, required intravenous fluid rehydration, dysentery (blood in stool), or required hospitalization. The enrollment was capped at 8-9 cases every two weeks in each age stratum at the sentinel health facility.

Exclusion criteria for case children with MSD: if a case child presented at the sentinel health facility but was currently enrolled as a case of MSD, meaning the child was previously enrolled in GEMS and still had a pending a follow-up visit.

Stool samples were taken at enrollment from case children and tested for a wide range of enteric pathogens. A full list of these pathogens can be found in Table 4. An enrolled child must have been capable of providing an adequate stool sample for microbiological analysis (>4 g) during a required time period to be included in the analysis.

For case children enrolled at health facilities, a clinical examination was carried out at enrollment by a licensed clinician or nurse employed by the study, anthropometric measurements were taken by trained study staff, and a risk factor questionnaire was also administered at this time.

At enrollment, caretakers of case children were given a Memory Aid form (see Appendix 2) and instructed on how to record the presence or absence of diarrhea for 14 days following enrollment. The form was designed to be suitable for adults regardless of literacy. Prior piloting of the Memory Aid had determined that it was acceptable to caretakers and at an appropriate literacy level.

A follow-up visit to all enrolled case children was carried out at home after 60 days. At the follow-up visit, a trained Community Interview interviewed the caretaker about the child's vital status, clinical condition, and interim illnesses, performed focused physical exams and anthropometric measurements, documented water, sanitation, and hygiene practices via interview and observation, and reviewed and collected the Memory Aid form. The Memory Aid forms were reviewed with the caretaker for unclear or incomplete responses.

Study Site

The rural western Kenya GEMS site is located within the Kenya Medical Research Institute/Centers for Disease Control and Prevention Health and Demographic Surveillance System (HDSS) study area near Lake Victoria in the Gem and Asembo districts of Nyanza Province. The Gem and Asembo HDSS represents an area of approximately 500 km^2 and a population of approximately 135,000 persons (population density approximately 300 persons per km^2), and is about 50-65 kilometers west of Kisumu city[69]. Information on pregnancies, births, deaths, and migration is collected in the HDSS every four months. Infant mortality in Nyanza Province is 95 per 1000 live births, and under five mortality is 149 per 1000 live births[70]. Among children, malaria is reported to be the most common cause of death in post-neonates, followed by anemia, dehydration and diarrhea, and pneumonia[69]. HIV prevalence for persons in the HDSS between the ages of 13 years and 34 years was 15.4% in 2003-2004[71].

Cryptosporidium Identification

Stool samples from all GEMS case children were tested in Kenya by enzyme immunoassay (EIA) for detection of *Cryptosporidium* oocyst antigens (TechLab®, Inc, VA, USA). DNA was extracted from a subset of stools that were EIA-positive for *Cryptosporidium*. Restriction fragment length polymorphism (RFLP) analyses of polymerase chain reaction (PCR) products were used to identify *Cryptosporidium* genotypes for these samples at the Waterborne Disease Prevention Branch, Molecular

Diagnostics Laboratory at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA. Stools were also tested for the other enteric pathogens described previously and listed in Table 4. Details on detection of all the enteric pathogens have been described elsewhere[68].

Anthropometric Measurements

Weight and height for each child was obtained at enrollment and at 60-day follow-up. Weight was recorded to the nearest 0.1 kilogram using scales that were calibrated weekly[67]. Recumbent length on a board was measured for children less than two years old; those older than 2 years were measured standing[67]. Weight-for-age, height-for-age, and weight-for-height z-scores were calculated from these measurements using a WHO SAS macro and the WHO Child Growth Standards as the reference population[72, 73]. For case children requiring rehydration, anthropometric measurements were measured again after rehydration. If the child was under observation for more than four hours, anthropometric measurements were obtained again upon discharge from the health facility. Of the measurements at enrollment, after rehydration, and at discharge, the last recorded anthropometric measurements were used as the baseline anthropometric measurements when comparing baseline measurements to 60-day follow-up measurements.

Data were cleaned for implausible height values. Values for height were set to missing if: a decrease in height of ≥ 1.5cm was recorded between baseline and follow-up, if an increase in height of >8cm was observed in children 0-6 months old in a follow-up visit occurring 50-60 days after initial height measurement, if an increase in height of >4cm was observed in children older than 6 months old in a follow-up visit occurring

50-60 days after initial height measurement, if an increase in height of >10cm was observed in children 0-6 months old in a follow-up visit occurring 61-90 days after initial height measurement, if an increase in height of >6cm was observed in children older than six months old in a follow-up visit occurring 61-90 days after initial height measurement, or if the absolute value of the height-for-age z-score was six or greater at enrollment or follow-up with a difference of three standard deviations between the two measurements.

Days of Diarrhea

For case children, the questionnaire administered at enrollment asked caretakers how many days the child had been experiencing diarrhea before presenting at the health facility. Data on days of diarrhea were obtained from the Memory Aid form for the 14 days after enrollment for GEMS case children. The total number of days was calculated as the sum of these two durations. For case children, a maximum of 21 days of diarrhea could be counted as the enrollment criteria stipulated that the child had to be enrolled within seven days of illness onset with MSD, and the Memory Aid captured 14 days post enrollment. The total days of diarrhea may include multiple episodes of diarrhea per child in the three-week period.

For analysis of the number of days of diarrhea prior to enrollment and in the two weeks following enrollment, data were restricted to those case children who had exactly one enteric pathogen isolated in their stool, also referred to henceforth as a single pathogen.

HIV Testing

For a sub-set of enrolled children, HIV status was determined by linking the GEMS data to data collected in the HDSS by the CDC Kenya Global AIDS Program which is part of the US President's Emergency Plan for AIDS Relief (PEPFAR). Data for case children were retrospectively linked to the Home-Based HIV Counseling and Testing (HBCT) Program, and was prospectively linked to Provider Initiated HIV Testing and Counseling (PITC). HBCT involves carrying out an HIV test at home, and the child is tested if the child's biological mother is determined to be HIV positive, or is deceased. PITC is HIV testing offered to all persons (and their relatives) attending health facilities regardless of their presenting conditions. Under both programs, persons identified as HIV positive are referred to HIV care and treatment. Children ≥18 months old had a rapid HIV antibody test and children <18 months old had a confirmatory PCR test.

For children not tested in HBCT we applied an HIV-negative result if their biological mother tested HIV-negative on or after the date the child was enrolled in GEMS. When individuals had HIV test results available via HBCT and PITC, the PITC data was reported.

Temperature and Rainfall

Daily maximum temperatures and millimeters of rainfall were obtained from the World Meteorological Organization World Weather Watch Program, maintained by the National Climatic Data Center, National Oceanic and Atmospheric Administration. The data station is located at the Kisumu Airport, in Kisumu city, Kenya which is 50-65 kilometers east of the HDSS study area.

Definitions

Surface water was defined as drinking water coming from a pond, lake, river, stream, dam, or earth pan. This is in accordance with the WHO UNICEF Joint Monitoring Program (JMP) standards except for the inclusion of earth pans which is western Kenya specific, thus grouped with dams in the GEMS survey but not explicitly mentioned in the JMP classification criteria[74].

Improved sanitation facilities are defined by the JMP as those that hygienically separate excreta from human contact[74]. JMP includes in its definition of improved sanitation facilities: flush or pour-flush toilets that connect to a piped sewer system, a septic tank, or a pit latrine, ventilated improved pit (VIP) latrines, pit latrines with slabs, and composting toilets[74]. The three types of sanitation facilities reported by GEMS Kenya respondents were traditional pit toilets, VIP latrines, and VIP latrines with water seals. For traditional pit toilets, information on slabs was not collected. Respondents who had a VIP latrine or a VIP latrine with a water seal were considered to have improved sanitation facilities. Those who reported having a traditional pit toilet or who did not have a facility for waste disposal were considered to have unimproved sanitation facilities.

Wealth index quintile was derived from a wealth index score that incorporated the number of rooms for sleeping in the house, whether a household had electricity, a television, a scooter or motorcycle, a radio, a bicycle, a car or truck, a telephone (mobile or non-mobile), a refrigerator, a finished floor (parquet or polished wood, vinyl or asphalt strips, ceramic tile, cement, or carpet), or a boat with a motor[75]. Principle component analysis was used to develop a wealth index to classify each household into one of five wealth index quintiles, representing the poorest to the wealthiest quintiles.

Breastfeeding: For breastfeeding, "partial" breastfeeding refers to children who are breastfed but also given food or liquid other than breast milk, whereas "exclusive" breastfeeding refers to giving children only breast milk without supplementing with other foods or liquids.

Underweight, Stunted, and Wasted were defined as having a weight-for-age z-score<-2, a height-for-age z-score<-2, and a weight-for-height z-score<-2 respectively. Children with a weight-for-age z-score<-3, height-for-age z-score<-3, and weight-for-height z-score<-3 were considered *severely underweight, severely stunted,* and *severely wasted* respectively.

Statistical Analysis

Data were analyzed in SAS 9.3 (SAS Institute, Inc., Cary, NC).

For the analysis of days of diarrhea, a Wilcoxon rank sum test was used to compare median days of diarrhea for case children with *Cryptosporidium* as a single pathogen to days of diarrhea for case children with a different single enteric pathogen.

Logistic regression was used for multivariable analyses. Two multivariable logistic regression models were created to assess characteristics associated with *Cryptosporidium* infection. In both models, children with a *Cryptosporidium* infection as well as those without a *Cryptosporidium* infection may have had multiple pathogens isolated in their stool.

The first model was an exploratory model to assess environmental, behavioral, and clinical characteristics (including enteric pathogen co-infections) associated with *Cryptosporidium* infections in GEMS case children. The full list of variables considered for inclusion in this model can be found in Appendix 1. Logistic regression using exact procedures was used to screen each variable for inclusion in this model: those variables with a p-value<0.10 were considered for inclusion in the multivariable logistic regression model. In this regression model, collinearity was assessed using conditional indexes. It was assumed that there were no collinearity problems in the model if all conditional indexes were less than 30. All two-way interactions were included in the model and a chunk test was used to assess whether interactions were significant. All interaction terms were dropped if the likelihood ratio test chi-square p-value was greater than 0.05. Backward elimination was then used to remove remaining variables until all variables remaining were significant at p<0.05. Confounding was not assessed as this model was not meant to assess causality.

A second model was used to assess potential risk factors associated with *Cryptosporidium* infection. For this model, clinical characteristics observed at enrollment were excluded from consideration because they temporally may not have preceded the *Cryptosporidium* infection and therefore could not be considered risk factors for *Cryptosporidium*. Other enteric pathogens isolated in stool were also excluded from consideration because it was not known whether children were infected with *Cryptosporidium* before, during, or after they were infected with the other enteric pathogens. Likewise, clinical signs and symptoms that the children experienced during their diarrheal episodes that were reported by caretakers at enrollment were not considered as it was also unclear whether these symptoms preceded or resulted from the *Cryptosporidium* infection. A full list of the variables considered for screening into this second model can be seen in Appendix 1 (indicated with asterisks). Screening for this model was performed in the same manner as it was for the first model and a collinearity assessment was also performed in the same way. For this model, gender and wealth index were considered as potential confounders. After the collinearity assessment, all two-way interactions between risk factors that screened into the model and the potential confounders were assessed using a chunk test as in the first model. Next, potential confounders were dropped from the model if their elimination did not result in a 10% or greater change in any of the coefficients on the exposure variables. All two-way interaction terms between exposure variables were assessed in a chunk test using likelihood ratios. Backward elimination was then used to remove exposure variables one by one until all remaining variables were significant at $p<0.05$.

For both adjusted and unadjusted analyses, Wald chi-square p-values and Wald 95% confidence intervals were reported. When unadjusted analyses were conducted, Fisher's exact p-values were reported when an expected cell count was less than five.

Ethical review

For the case-control study, written informed consent was obtained in the local dialect (Dholuo) from all participating caretakers before enrollment. The study protocols for case-control study, inclusive of the linking to HIV status data collected by the CDC Global AIDS Program were reviewed and approved by the Scientific and Ethical Review Committees of the Kenya Medical Research Institute (KEMRI Protocol # 1155) and the Institutional Review Board (IRB) of the University of Maryland, School of Medicine, Baltimore, MD, USA (UMD Protocol # H-28327). The IRB for the Centers for Disease Control and Prevention, Atlanta, GA, USA deferred its review to the University of Maryland IRB (CDC Protocol # 5038).

RESULTS

Demographics

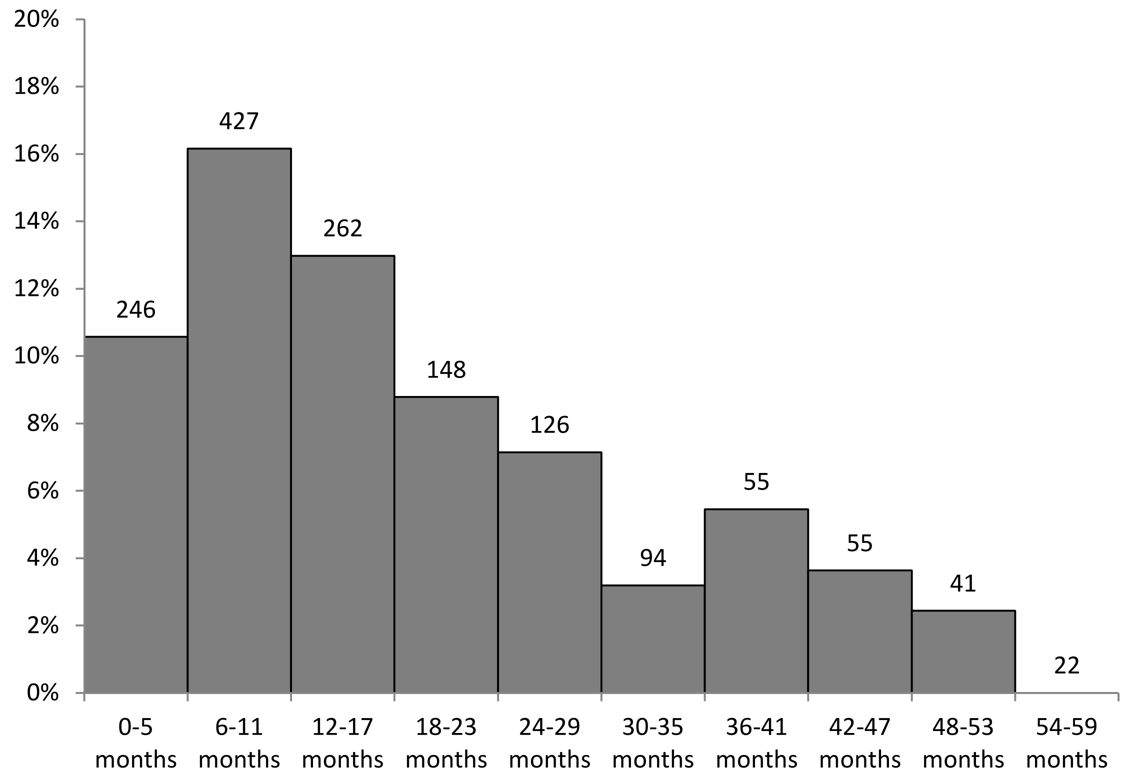
GEMS enrolled 1,476 case children at the rural western Kenya site between January 31, 2008 and January 28, 2011. Of the 1,476 case children, 160 (10.8%) had a *Cryptosporidium* infection identified. Descriptive statistics for these case children can be seen in Table 1. Approximately 43% of the enrolled case children were female. Approximately 46% of enrolled GEMS case children were infants under the age of 12 months. There was a higher proportion of infants among those case children with a *Cryptosporidium* infection than among those without a *Cryptosporidium* infection. The percentage of case children who had a *Cryptosporidium* infection is shown in six-month categories in Figure 1 to further explore the age pattern of *Cryptosporidium* infections in case children. The primary caretaker for most children was the biological mother. Most caretakers had an education of less than primary school or had completed primary school but had not had further schooling. The proportion of children falling in each wealth index quintile was similar between case children with and without a *Cryptosporidium* infection, also shown in Table 1. Almost all case children (99.5%) came from compounds in which an animal was present. The median number of people sleeping in a case child's household was four.

Table 1. Demographics of GEMS Kenya Case Children

	GEMS Kenya Case Children	
	with *Cryptosporidium* (N=160)	without *Cryptosporidium* (N=1,316)
Gender		
Female	64 (40.0%)	573 (43.5%)
Age		
0-11 months	95 (59.4%)	578 (43.9%)
12-23 months	47 (29.4%)	363 (27.6%)
24-59 months	18 (11.3%)	375 (28.5%)
Primary Caretaker		
Mother	153 (95.6%)	1,264 (96.0%)
Father	3 (1.9%)	6 (0.5%)
Grandmother	3 (1.9%)	29 (2.2%)
Other	1 (0.6%)	17 (1.3%)
Education of Primary Caretaker		
No formal schooling	2 (1.3%)	20 (1.5%)
Less than primary school	81 (50.6%)	568 (43.1%)
Completed primary school	72 (45.0%)	640 (48.6%)
Completed secondary school	5 (3.1%)	77 (5.9%)
Had post-secondary school	0	9 (0.7%)
Religious education only	0	1 (0.1%)
Don't know	0	1 (0.1%)
Wealth Index Quintile		
First quintile (poorest)	30 (18.8%)	223 (16.9%)
Second quintile (poor)	28 (17.5%)	298 (22.6%)
Third quintile (middle)	39 (24.4%)	338 (25.7%)
Fourth quintile (wealthy)	26 (16.3%)	205 (15.6%)
Fifth quintile (wealthiest)	37 (23.1%)	252 (19.1%)
Household Characteristics		
Presence of animal in compound[a]	157 (98.1%)	1,311 (99.6%)
Median number of people sleeping in house	4	4

a. See Appendix 1 for full list of animals

Figure 1. Percent of GEMS Kenya Case Children with *Cryptosporidium* and Total Enrollment Numbers, by Age



Note: the numbers above bars denote total GEMS Kenya case children enrolled in each age category

Clinical Signs and Symptoms during MSD Episode

In the enrollment questionnaire, caretakers were asked about clinical signs and symptoms that case children had experienced during their illness. The most common signs and symptoms reported for case children with *Cryptosporidium* were being very thirsty (83%), fever (72%), being irritable or restless (72%), abdominal pain (64%), cough (58%), decreased activity or lethargy (58%), and vomiting (49%). Clinical signs and symptoms reported were similar for children with and without a *Cryptosporidium* infection. Clinical signs of malnutrition were also recorded at the health facility at enrollment and are shown in Table 2.

Table 2. Clinical Signs and Symptoms among GEMS Kenya Case Children

	GEMS Kenya Case Children	
	with *Cryptosporidium* (N=160)	without *Cryptosporidium* (N=1,316)
Clinical Symptoms During MSD Episode Reported by Caretakers at Enrollment		
Very thirsty	132 (83.0%)	1,100 (84.1%)
Fever[a]	115 (71.9%)	1,023 (77.7%)
Irritable or restless	115 (71.9%)	902 (68.6%)
Abdominal pain	100 (64.1%)	852 (65.7%)
Cough	93 (58.1%)	816 (62.0%)
Decreased activity or lethargy	93 (58.1%)	713 (54.2%)
Vomiting ≥3 times per day	79 (49.4%)	638 (48.5%)
Drank much less than usual	42 (26.3%)	305 (23.2%)
Difficulty breathing	27 (16.9%)	273 (20.7%)
Loss of consciousness	18 (11.3%)	125 (9.5%)
Blood in stools	10 (6.3%)	148 (11.3%)
Rectal straining	9 (5.7%)	119 (9.2%)
Unable to drink	6 (3.8%)	57 (4.3%)
Convulsion	2 (1.3%)	26 (2.0%)
Rectal prolapse	1 (0.6%)	16 (1.2%)
Clinical Features of Malnutrition Observed during Physical Examination at Enrollment		
Flaky skin	10 (6.3%)	35 (2.7%)
Abnormal hair	10 (6.3%)	66 (5.0%)
Undernourishment	21 (13.1%)	115 (8.7%)
Bipedal edema	2 (1.3%)	20 (1.5%)

a. Perceived by caretaker or measured at ≥38°C

Stool Sample Consistency and Characteristics, and Co-Infections Identified in Stool

Characteristics of the stool samples that case children produced at enrollment are shown in Table 3. Blood and pus were rarely reported in the stool of case children (both with and without a *Cryptosporidium* infection). Mucus in stool was common in both case children with *Cryptosporidium* (76.3%) and without *Cryptosporidium* (67.7%). Watery stool ("opaque watery" or "rice water/clear watery") was common in case children with and without *Cryptosporidium* and was seen somewhat more often in the stool of case children with a *Cryptosporidium* infection.

The majority (72.5%) of those case children with a *Cryptosporidium* infection had a co-infection with one or more other enteric pathogens. Among the case children who did not have a *Cryptosporidium* infection, 21.6% did not have any pathogens identified, 42.1% had one enteric pathogen identified, and 36.3% had multiple pathogens identified. A full list of pathogens identified among GEMS Kenya case children is shown in Table 4. Case children with *Cryptosporidium* had similar pathogens identified as case children without *Cryptosporidium*, except that a lower proportion of case children with *Cryptosporidium* identified had rotavirus or *Shigella* concurrently detected in their stool.

Table 3. Stool Characteristics and Number of Pathogens Identified in Stool of GEMS Kenya Case Children

	GEMS Kenya Case Children	
	with *Cryptosporidium* (N=160)	without *Cryptosporidium* (N=1,316)
Stool Characteristics		
Blood in stool	4 (2.5%)	47 (3.6%)
Mucus in stool	122 (76.3%)	891 (67.7%)
Pus in stool	7 (4.4%)	44 (3.3%)
Stool Consistency		
Formed	2 (1.3%)	32 (2.4%)
Soft	5 (3.1%)	86 (6.5%)
Thick liquid	57 (35.6%)	512 (38.9%)
Opaque watery	81 (50.6%)	625 (47.5%)
Rice water/clear watery	15 (9.4%)	61 (4.6%)
Number of Pathogens Isolated in Stool		
0	0[a]	284 (21.6%)
1	44 (27.5%)	554 (42.1%)
2	73 (45.6%	370 (28.1%)
3	34 (21.3%)	94 (7.1%)
4	8 (5.0%)	13 (1%)
5	1 (0.6%)	1 (0.1%)

a. All children with *Cryptosporidium* had at least one pathogen (namely, *Cryptosporidium*) isolated in their stool

Table 4. Enteric Pathogens Identified in Stool of GEMS Kenya Case Children

	GEMS Kenya Case Children	
	with *Cryptosporidium* (N = 160)	without *Cryptosporidium* (N = 1,316)
Enteric Pathogen		
Giardia	29 (18.1%)	252 (19.1%)
Enteroaggregative *E. coli*	27 (16.9%)	190 (14.4%)
C. jejuni	19 (11.9%)	114 (8.7%)
Enterotoxigenic *E. coli* (ST or ST/LT)	15 (9.4%)	120 (9.1%)
Typical enteropathogenic *E. coli*	13 (8.1%)	103 (7.8%)
Atypical enteropathogenic *E. coli*	10 (6.3%)	80 (6.1%)
Enterotoxigenic *E. coli* (LT only)	9 (5.6%)	82 (6.2%)
Rotavirus	8 (5.0%)	209 (15.9%)
Non-typhoidal *Salmonella*	6 (3.8%)	75 (5.7%)
Norovirus GI	6 (3.8%)	34 (2.6%)
C. coli	5 (3.1%)	68 (5.2%)
Sapovirus	5 (3.1%)	35 (2.7%)
Norovirus GII	4 (2.5%)	59 (4.5%)
Adenovirus (type 40/41)	4 (2.5%)	29 (2.2%)
Astrovirus	4 (2.5%)	20 (1.5%)
Shigella	3 (1.9%)	108 (8.2%)
Adenovirus (not type 40/41)	2 (1.3%)	33 (2.5%)
E. histolytica	0*	14 (1.1%)
V. cholera O1	0*	7 (0.5%)
Aeromonas	0*	1 (0.1%)
Typhoidal *Salmonella*	0*	0*
Enterohemorrhagic *E. coli*	0*	0*

*tested for; not identified
note: coinfections possible

Cryptosporidium Sub-Typing

DNA was extracted from a random subset of 64 (40%) of the 160 *Cryptosporidium*-positive stool samples from GEMS Kenya case children. Nested 18S PCR detected *Cryptosporidium* in 43 (67%) of these specimens, which is within the expected yield range when accounting for the freeze-thaw cycles during testing. Of the 43 specimens, 35 (81%) were of the genotype *C. hominis* and 14% were *C. parvum*. *C. meleagridis* and *C. canis* were found in one specimen each.

Days of Diarrhea

Among GEMS case children with a single enteric pathogen identified in their stool who had information for the number of days of diarrhea prior to enrollment and in the two weeks following enrollment, those with *Cryptosporidium* had a median of nine total days of diarrhea and an average of 10.1 days (range: 4-18 days) out of a maximum possible 21 days captured (see Table 5 and Figure 2).

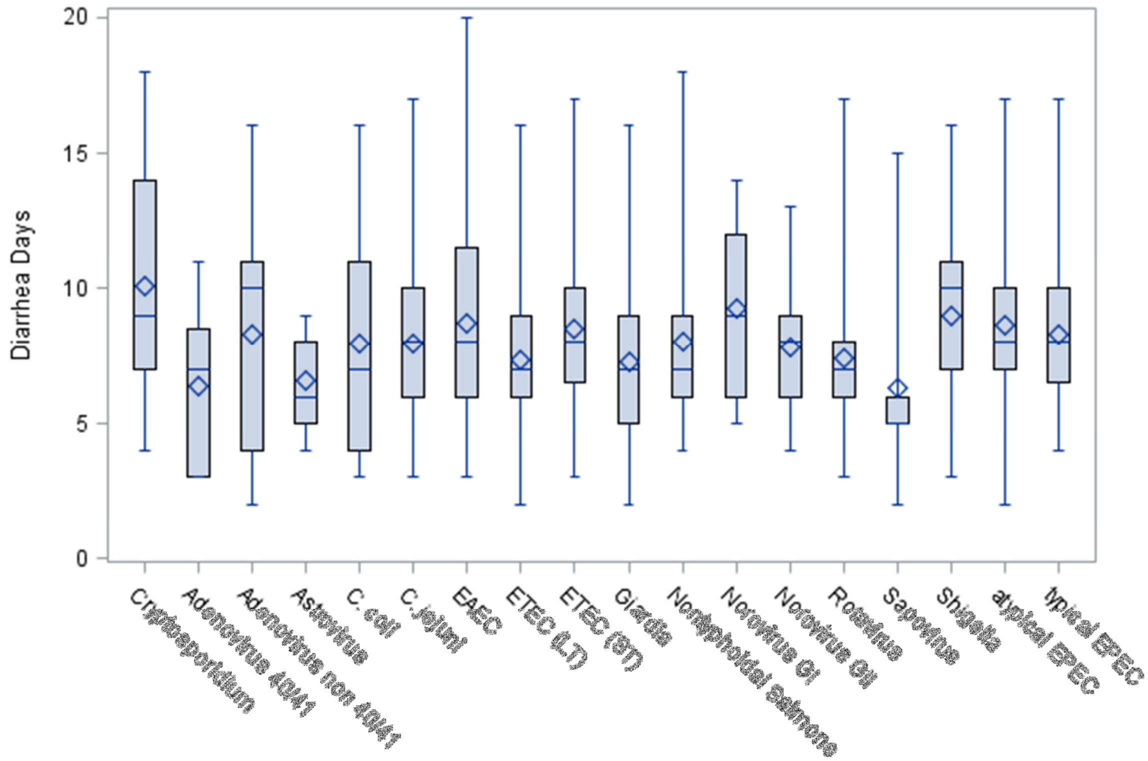
The highest average number of days of diarrhea among case children with a single pathogen isolated in their stool was seen in children with a *Cryptosporidium* infection. The highest median number of days of diarrhea was seen in case children with adenovirus (non-type 40/41) and *Shigella* (10 days), followed by case children with *Cryptosporidium* and norovirus type GI (9 days). Among these children, the median days of diarrhea experienced by those with *Cryptosporidium* was significantly higher than the median number of days of diarrhea experienced by those case children with a rotavirus, enterotoxigenic *E. coli* (LT only), or *Giardia* infection, accounting for multiple comparisons.

Table 5. Days of Diarrhea Experienced by GEMS Kenya Case Children with a Single Enteric Pathogen (Prior to Enrollment and in 14-Day Follow-Up)

	Days of Diarrhea			
	case children with this single pathogen	Average	Median	Wilcoxon p-value for comparison of medians[a]
Enteric Pathogen				
Cryptosporidium	39	10.1	9.0	ref.
Norovirus GI	7	9.3	9.0	0.78
Shigella	35	9	10.0	0.51
enteroaggregative *E. coli*	56	8.7	8.0	0.08
atypical enteropathogenic *E. coli*	17	8.6	8.0	0.26
enterotoxigenic *E. coli* (ST)	36	8.5	8.0	0.12
Adenovirus (not type 40/41)	9	8.3	10.0	0.37
typical enteropathogenic *E. coli*	40	8.3	8.0	0.06
non-typhoidal *Salmonella*	31	8	7.0	**0.03**
C. jejuni	33	7.9	8.0	**0.04**
C. coli	15	7.9	7.0	0.08
Norovirus GII	13	7.8	8.0	0.09
Rotavirus	64	7.4	7.0	**<0.01***
enterotoxigenic *E. coli* (LT)	26	7.3	7.0	**<0.01***
Giardia	85	7.3	7.0	**<0.01***
Astrovirus	7	6.6	6.0	**0.03**
Adenovirus (type 40/41)	8	6.4	7.0	**0.03**
Sapovirus	9	6.3	5.0	**0.02**
E. histolytica	4	6.5	6.5	n/a[b]
V. cholera O1	1	3.0	3	n/a[b]

a. p-value from Wilcoxon rank sum test; bolded values were significant at $p<0.05$
those with an asterisk (*) remain significant ($p<0.05$) when a correction is made for multiple tests using the false discovery rate method
b. comparisons only made for those single pathogens isolated in the stool of at least 5 GEMS Kenya case children

Figure 2. Days of Diarrhea Experienced by GEMS Kenya Case Children with a Single Enteric Pathogen (Prior to Enrollment and in 14-Day Follow-Up)



Anthropometric Measurements as Indicators of Malnutrition

Anthropometric indicators of malnutrition are summarized in Table 6 for GEMS Kenya case children who had both baseline and follow-up anthropometric measurements available. At baseline, the proportions of children who were underweight, stunted, wasted, severely underweight, severely stunted, and severely wasted were not significantly different between children with and without a *Cryptosporidium* infection. At the 60-day follow-up subsequent to their diarrheal episode, children who had been enrolled with a *Cryptosporidium* infection were approximately twice as likely to be underweight (OR=2.0, p=0.0004), wasted (OR=2.1, p=0.0033), or severely underweight (OR=1.8, p=0.0383) compared to those without a *Cryptosporidium* infection. The proportions of GEMS case children with a *Cryptosporidium* infection who were underweight, stunted, wasted, severely underweight, severely stunted, and severely wasted were all higher at follow-up than at baseline.

Table 6. Anthropometric Indicators of Malnutrition for GEMS Kenya Case Children with and without *Cryptosporidium* Infection at Baseline and 60-Day Follow-Up

	GEMS Kenya Case Children		
	with *Cryptosporidium* (N = 145)[a]	without *Cryptosporidium* (N = 1,218)[a]	Odds Ratio[b] (95% CI)
Enrollment			
Underweight	25 (17%)	207 (17%)	1.0 (0.6, 1.6)
Stunted	37 (26%)	334 (27%)	0.9 (0.6, 1.3)
Wasted	10 (7%)	88 (7%)	1.0 (0.5, 1.9)
Severely Underweight	11 (7.6%)	59 (4.8%)	1.6 (0.8, 3.1)
Severely Stunted	14 (9.7%)	111 (9.1%)	1.1 (0.6, 1.9)
Severely Wasted	4 (2.8%)	24 (2.0%)	1.4 (0.5, 4.1)
60-Day Follow-Up			
Underweight	45 (31%)	225 (18%)	**2.0 (1.4, 2.9)**
Stunted	56 (39%)	419 (34%)	1.2 (0.8, 1.7)
Wasted	22 (15%)	95 (8%)	**2.1 (1.3, 3.5)**
Severely Underweight	17 (11.7%)	84 (6.9%)	**1.8 (1.0, 3.1)**
Severely Stunted	17 (11.7%)	159 (13.0%)	0.9 (0.5, 1.5)
Severely Wasted	8 (5.5%)	34 (2.8%)	2.0 (0.9, 4.5)

a. includes only those with anthropometric measurements available at baseline and at 60-day follow-up
b. referent: GEMS Kenya case children without *Cryptosporidium*

Hospitalizations and Deaths

There was no significant difference in the proportion of GEMS Kenya case children with and without a *Cryptosporidium* infection who were hospitalized (12.5% vs. 10.6%, p=0.46). Of the 152 children with a *Cryptosporidium* infection who had available follow-up information, 4.6% had died by the time of the 60-day follow-up compared to 3.6% of case children without *Cryptosporidium*. The difference was not statistically significant (p=0.51). Of the seven case children with a *Cryptosporidium* infection who died, 4 (57.1%) died in a health facility. Of the 45 case children without a *Cryptosporidium* infection who died, 15 (33.3%) died in a health facility. This difference was also not statistically significant (Fisher's exact test p-value=0.40). For case children who died by the time of the 60-day follow-up, the median time from enrollment to death was 15 days for those with a *Cryptosporidium* infection and 12 days for those without a *Cryptosporidium* infection.

Table 7. Hospitalizations and Deaths among GEMS Kenya Case Children

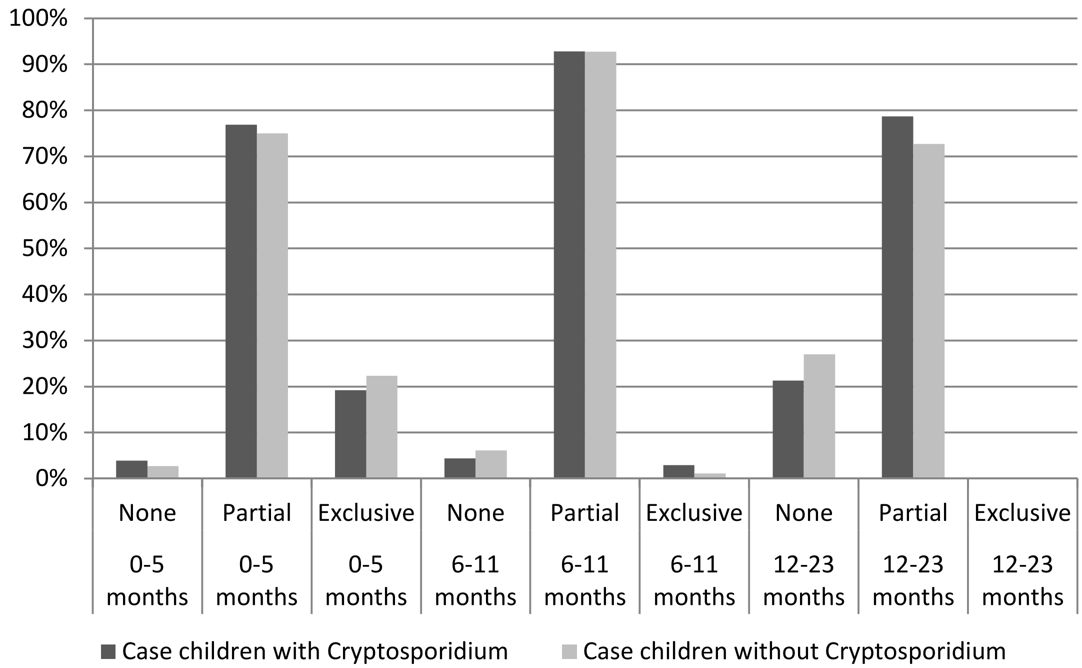
	GEMS Kenya Case Children	
	with *Cryptosporidium* (N=160)	without *Cryptosporidium* (N=1,316)
Hospitalized at Enrollment	20 (12.5%)	139 (10.6%)
Had 60-day follow-up information	152 (95.0%)	1,267 (96.3%)
Died by 60-day follow-up[a]	7 (4.6%)	45 (3.6%)
Died in a health facility[b]	4 (57.1%)	15 (33.3%)

a. denominator is GEMS Kenya case children with follow-up information
b. denominator is GEMS Kenya case children who died

Breastfeeding

Figure 3 below shows the percentage of case children who were exclusively breastfed, partially breastfed, or not breastfed for ages 0-5 months, 6-11 months, and 12-23 months. Most children in all three age groups were partially breastfed. Twenty-two percent (22%) of case children less than six months old were exclusively breastfed. Exclusive breastfeeding was uncommon past the age of six months. There was no significant difference in breastfeeding practices between case children with and without a *Cryptosporidium* infection among those less than 24 months old within any of the age strata shown in Figure 3 (0-5 months, 6-11 months, 12-23 months; p>0.05 in all strata). Of note, there were seven exclusively breastfed case children who had a *Cryptosporidium* infection.

Figure 3. Breastfeeding Practices among GEMS Kenya Case Children Less than Two Years Old



HIV Status

HIV status was available for almost half (48%) of GEMS Kenya case children. Among children with test results available, 4.2% of children with *Cryptosporidium* were HIV-positive, compared to 2.7% of case children without a *Cryptosporidium* infection.

Table 8. HIV Status of GEMS Kenya Case Children

	GEMS Kenya Case Children	
	with *Cryptosporidium* (N=160)	without *Cryptosporidium* (N=1,316)
Had HIV test result available	72 (45.0%)	637 (48.4%)
HIV Positive[a]	3 (4.2%)	17 (2.7%)

a. denominator is those with test result available

Water, Sanitation, and Hygiene Characteristics

Data on the water, sanitation, and hygiene characteristics of the households of GEMS Kenya case children with and without a *Cryptosporidium* infection are shown in Table 9. When the caretaker was asked what the primary water source was in each case child's household in the two weeks prior to enrollment, the most common responses were: rainwater (25.6% of those with *Cryptosporidium,* 37.1% of those without *Cryptosporidium*), river or stream (25.0% of those with *Cryptosporidium*, 25.2% of those without *Cryptosporidium*), and borehole (10.6% of those with *Cryptosporidium,* 10.4% of those without *Cryptosporidium*). Patterns of usage of rainwater and surface water, the two predominate sources of drinking water among the GEMS Kenya case children, are displayed in relation to monthly rainfall in Figure 4, Figure 5, and Figure 6 (see "Additional Figures" on page 56). The proportion of households using rainwater as a main source of drinking water often increased around the months of the year with the highest rainfall; however, rainwater use did not always increase or decrease with precipitation, perhaps because of the high proportion (91%) of households that reported using stored drinking water. A lower proportion of case children with a *Cryptosporidium* infection came from households using rainwater as a primary drinking water source compared to case children who did not have a *Cryptosporidium* infection (25.6% vs. 37.1%). More than half of the GEMS Kenya case children came from households that reported regularly treating drinking water (58.8% of those with a *Cryptosporidium* infection and 62.6% of those without a *Cryptosporidium* infection). The two most common treatments for drinking water were chlorination and boiling (see Table 9).

The most common type of feces disposal facility among GEMS Kenya case children was a traditional pit toilet (reported by 80.0% of those with *Cryptosporidium* and 73.5% of those without *Cryptosporidium*).

Most respondents reported that they washed their hands before eating and after defecating. Less than half of respondents reported handwashing at each of the following times: before cooking, before nursing or preparing a baby's food, after cleaning a child who had defecated, or after handling domestic animals. Reported handwashing was similar among case children with and without *Cryptosporidium*, although fewer caretakers of children with a *Cryptosporidium* infection reported regularly washing their hands after defecating (70.6% of caretakers of case children with a *Cryptosporidium* infection compared to 77.1% of caretakers of case children without a *Cryptosporidium* infection; Table 9).

Table 9. Water, Sanitation, and Hygiene Characteristics, GEMS Kenya Case Children

	GEMS Kenya Case Children with *Cryptosporidium* (N=160)	GEMS Kenya Case Children without *Cryptosporidium* (N=1,316)
Primary Source of Drinking Water[a]		
Rainwater	41 (25.6%)	488 (37.1%)
River or stream	40 (25.0%)	332 (25.2%)
Borehole	17 (10.6%)	137 (10.4%)
Pond or lake	13 (8.1%)	93 (7.1%)
Public tap	10 (6.3%)	43 (3.3%)
Open public well	8 (5.0%)	50 (3.8%)
Unprotected spring	8 (5.0%)	48 (3.6%)
Protected spring	8 (5.0%)	43 (3.3%)
Shallow tube well	5 (3.1%)	36 (2.7%)
Deep tube well	3 (1.9%)	24 (1.8%)
Covered public well	2 (1.3%)	8 (0.6%)
Covered well in house or yard	1 (0.6%)	5 (0.4%)
Open well in house or yard	1 (0.6%)	3 (0.2%)
Dam or earth pan	1 (0.6%)	3 (0.2%)
Piped into yard	1 (0.6%)	2 (0.2%)
Bought	1 (0.6%)	1 (0.1%)
Water Treatment and Storage		
Usually Treat Drinking Water	94 (58.8%)	824 (62.6%)
Chlorine	74 (78.7%)[b]	569 (69.1%)
Boiling	9 (9.6%)[b]	133 (16.1%)
PUR	6 (6.4%)[b]	68 (8.3%)
Filtered through Cloth	4 (4.3%)[b]	42 (5.1%)
Alum	1 (1.1%)	8 (1.0%)
Filter (not cloth)	0	1 (0.1%)
Other chemical	0	1 (0.1%)
Gave Child Stored Water in Last 2 Weeks	145 (90.6%)	1,199 (91.1%)
Household facility for feces disposal		
Traditional pit toilet	128 (80.0%)	967 (73.5%)
Ventilated improved pit (VIP) latrine	3 (1.9%)	86 (6.5%)
No facility	29 (18.1%)	262 (19.9%)
Other	0 (0.0%)	1 (0.1%)
Handwashing		
Before eating	131 (81.9%)	1,109 (84.3%)
After defecating	113 (70.6%)	1,014 (77.1%)
Before nursing/preparing baby's food	45 (28.1%)	365 (27.7%)
Before cooking	44 (27.5%)	438 (33.3%)
After cleaning a child who defecated	43 (26.9%)	294 (22.3%)
After handling domestic animals	11 (6.9%)	110 (8.4%)

a. in the two weeks prior to enrollment
b. denominator: those case children whose caretakers reported usually treating drinking water

Characteristics Associated with Cryptosporidium Infections

A full list of variables that were considered for screening in the multivariable logistic regression models can be found in Appendix 1. Having a *Shigella* infection and having an improved sanitation facility were both negatively associated with having a *Cryptosporidium* infection in GEMS case children in unadjusted analyses performed for screening; however, they were not considered for inclusion in the final model because they occurred in fewer than five case children with *Cryptosporidium* (see "Methods"). Breastfeeding and use of milk as a home treatment for diarrhea could not be assessed because of their collinearity with age category.

The results of the logistic regression model are shown in Table 10.

Case children with *Cryptosporidium* were significantly more likely to be younger children between the ages of 0-11 months and 12-23 months, compared to case children without a *Cryptosporidium* infection ($p<0.001$).

Case children with a *Cryptosporidium* infection were significantly less likely to have a rotavirus infection compared to case children without *Cryptosporidium* (OR=0.20, $p<0.001$). Case children with a *Cryptosporidium* infection were significantly more likely to have had flaky skin at enrollment (OR=2.6, $p=0.013$) or to have provided a watery stool sample at enrollment (OR=1.44, $p=0.036$) compared to case children without a *Cryptosporidium* infection.

Case children with a *Cryptosporidium* infection were significantly less likely to have come from homes that reported using rainwater as a main source of drinking water (OR=0.58, p=0.005) or from homes in which the caretaker reported boiling of drinking water (OR=0.49, p = 0.045), compared to children without a *Cryptosporidium* infection.

Table 10. Characteristics Significantly Associated with *Cryptosporidium* Infection in GEMS Case Children

Characteristic	Adjusted Odds Ratio (95% Confidence Interval)	Adjusted p-value
Having a rotavirus infection	0.20 (0.09, 0.41)	<0.001
Main source of drinking water is rainwater[a]	0.58 (0.39, 0.84)	0.005
Having flaky skin at enrollment	2.61 (1.22, 5.56)	0.013
Watery stool at enrollment	1.44 (1.02, 2.03)	0.036
Caretaker reported boiling drinking water	0.49 (0.24, 0.98)	0.045
Age category		<0.001
0-11 months	3.60 (2.2, 5.93)	
12-23 months	2.82 (1.63, 4.87)	
24-59 months	ref	

a. main source of drinking water in the two weeks prior to enrollment, as reported by caretaker

Potential Risk Factors for *Cryptosporidium*

A full list of variables that were considered for screening for the multivariable logistic regression model to assess potential risk factors for *Cryptosporidium* infection can be found in Appendix 1 (indicated with asterisks). As in the previous model, having an improved sanitation facility was not considered for inclusion because only three case children with *Cryptosporidium* had an improved sanitation facility (see Methods). Breastfeeding could not be assessed because of its collinearity with age category. Wealth index quintile was considered to be a significant confounder for this analysis. Gender was also considered as a confounder but did not remain in the final model.

The full list of potential risk factors associated with *Cryptosporidium* in GEMS case children can be seen in Table 11, which is adjusted for wealth.

The potential risk factors associated with *Cryptosporidium* infection in GEMS case children in this model included being in the two younger age categories (0-11 months or 12-23 months), coming from a home using a main source of drinking water other than rainwater, coming from a home in which caretakers did not report boiling water, and coming from a home in which caretakers did not report washing hands after defecating.

Table 11. Potential Risk Factors Associated with *Cryptosporidium* Infection in GEMS Case Children, Controlling for Wealth Index Quintile

Characteristic	Adjusted Odds Ratio (95% Confidence Interval)	Adjusted p-value
Main source of drinking water is rainwater[a]	0.61 (0.42, 0.89)	0.010
Caretaker reports usually washing hands after defecating	0.69 (0.47, 1.00)	0.048
Caretaker reported boiling drinking water	0.48 (0.24, 0.97)	0.041
Age category		<0.001
0-11 months	3.02 (1.84, 4.94)	
12-23 months	2.57 (1.49, 4.41)	
24-59 months	ref	

a. main source of drinking water in the two weeks prior to enrollment, as reported by caretaker

DISCUSSION AND RECOMMENDATIONS

Our study identified that *Cryptosporidium* caused serious infection in young children and concerning adverse consequences subsequent to the illness. There were several notable findings of this analysis. First, clinical signs and symptoms experienced by GEMS children with moderate-to-severe diarrhea during their illness (and before reporting to a health facility) were similar for those with and without a *Cryptosporidium* infection. Second, case children with *Cryptosporidium* were significantly more likely than those without *Cryptosporidium* to be underweight or wasted at 60-day follow-up. Third, children with *Cryptosporidium* infections had a high number of days of diarrhea prior to enrollment and in the 14 days after enrollment (median nine days). Finally, in a multivariable logistic regression model looking at characteristics associated specifically with *Cryptosporidium* in GEMS Kenya case children, being a young child (less than two years old), producing watery stool at enrollment, and having flaky skin at enrollment were significantly positively associated with having a *Cryptosporidium* infection, whereas having a rotavirus infection, coming from a household that reported boiling of drinking water, and coming from a household that reported using rainwater as a main source of drinking water were negatively associated with *Cryptosporidium* infection. In a separate model specifically considering the potential risk factors, controlling for wealth, again we found that being a younger child (less than two years old) was positively associated with *Cryptosporidium*, and coming from a household that reported boiling drinking water and coming from a household that reported using rainwater as a main source of drinking water were negatively associated with *Cryptosporidium* infection. In the risk factor model, coming from a household in which the caretaker reported washing hands after defecating was also negatively associated with *Cryptosporidium* infection.

Age

Our results from GEMS Kenya show a similar age pattern of *Cryptosporidium* infections as has been previously reported in sub-Saharan Africa. In a review of cryptosporidiosis in sub-Saharan Africa, Mor *et al.* reported that *Cryptosporidium* cases in children peak between the ages of 6 and 12 months and drop off after that age[47], which is consistent with our findings.

The high prevalence of cryptosporidiosis among children less than two years old found in our study, coupled with other research that shows long-term effects of *Cryptosporidium* infections and diarrhea early in life (see "Literature Review"), underscores the need for preventive measures aimed at households with infants and young children, as well as improved diarrhea case management.

Early childhood diarrheal infections have been predictive of decreased cognitive function and physical fitness later in childhood[49], as well as decreased readiness for school and lower performance in school[76]. A study in Brazil found that *Cryptosporidium* in the first two years of life was associated with physical fitness deficits for several years after the infection[49]. Studies in Brazil and Peru showed that *Cryptosporidium* infections in the first year of life are associated with an increased burden of acute diarrhea for approximately two years after the diagnosis of *Cryptosporidium*[18] and with growth shortfalls that are more pronounced than those who have an infection later in childhood[16]. The increase in diarrheal disease burden after cryptosporidiosis may be due to intestinal injury leading to malabsorption or increased susceptibility to other enteric pathogens[18]. In one study, children with *Cryptosporidium* had significantly higher lactulose-mannitol ratios during their diarrheal episode compared to control children, indicating increased intestinal permeability to pathogens and/or a reduced intestinal absorption capacity for nutrients. The effects, however, were short-term[77]. Intestinal injury and effects on growth from cryptosporidiosis may lead to malabsorption of antiretroviral or antituberculous drugs[21].

In Kotloff *et al.*'s analysis of major pathogens in GEMS, rotavirus had the highest attributable fraction for MSD episodes among infants at the Kenya site, followed by *Cryptosporidium*[2]. As rotavirus vaccinations become available, a focus should be on preventive measures and treatment for *Cryptosporidium*.

Much of the research on longer-term effects of childhood *Cryptosporidium* infections on growth faltering and development has been conducted in South America. Follow-up beyond 60 days of the GEMS Kenya case children with *Cryptosporidium* infections could provide valuable information on the effects of having a *Cryptosporidium* infection early in life that would be more regionally specific.

Clinical Signs and Symptoms and Days of Diarrhea

Clinical signs and symptoms reported by caretakers that occurred during case children's diarrheal illnesses prior to enrollment were similar for children with and without *Cryptosporidium*. At enrollment, having flaky skin and producing a watery stool sample were positively associated with *Cryptosporidium* infection in GEMS Kenya case children.

By the time of 60-day follow-up, approximately 5% of case children who had a *Cryptosporidium* infection at baseline had died. This proportion was higher than the proportion of children with moderate-to-severe diarrhea without a *Cryptosporidium* who had died, although the results were not statistically significant. Other studies, however, have shown *Cryptosporidium* to be associated with excess mortality for children who become infected in infancy[17] and early childhood[15]. Additional follow-up of GEMS Kenya children with moderate-to-severe diarrhea could examine whether excess mortality occurred in children with *Cryptosporidium* beyond the 60-day follow-up window.

The fact that children with MSD who had a *Cryptosporidium* infection had similar signs and symptoms during the diarrheal episode to other children with MSD underscores the need for a reliable and readily-available rapid diagnostic test for *Cryptosporidium* coupled with routine testing in clinics in Kenya. Early diagnosis and management of cryptosporidiosis may mitigate growth deficits or reduce deaths. The excess days of diarrhea and relatively high 60-day mortality of case children with moderate-to-severe diarrhea further emphasize the need for available treatment for children with cryptosporidiosis, as well as future research on treatment of cryptosporidiosis for immunocompromised individuals.

Malnutrition

At baseline, 17% of case children with a *Cryptosporidium* infection were underweight and 7% were wasted. These proportions were the same among children without a *Cryptosporidium* infection. At 60-day follow-up, among the same group of case children who had *Cryptosporidium* infections at baseline, 31% were underweight and 15% were wasted, whereas for children without cryptosporidiosis, similar proportions of children were underweight and wasted at baseline as at follow-up. This nearly doubling in the proportion of children with *Cryptosporidium* who were underweight and wasted may be a result of fluid and weight loss resulting from many days of diarrhea. Asymptomatic *Cryptosporidium* infections in children can also be associated with decreased growth[48], indicating that diarrhea may not be the only cause of growth deficits and that other factors, such as changes to intestinal architecture leading to malabsorption of nutrients, may also be involved. There was also an increase from baseline to follow-up in the proportion of children who had a *Cryptosporidium* infection at baseline who were stunted. This was also true of case children who did not have a *Cryptosporidium* infection, although the difference was less pronounced. One study of cryptosporidiosis in children reported that there was catch-up weight gain that was usually complete by six months; however, deficiencies in linear growth were not always recovered[16]. In a review of child undernutrition, stunting among children in low- and middle-income countries was reported to predict decreased performance in school or cognitive tests and has additionally been related to lower earnings later in life[78].

Breastfeeding

Breastfeeding is thought to be protective against *Cryptosporidium* infection[36]. In our multivariable logistic regression models, we were not able to assess the association between breastfeeding and *Cryptosporidium* because of the collinearity between breastfeeding and age. However, in unadjusted analyses, there was no significant association between *Cryptosporidium* and degree of breastfeeding (no breastfeeding, partial breastfeeding, or exclusive breastfeeding) within the age strata considered

(0-5 months, 6-11 months, and 12-23 months). The majority of case children less than two years old in our study population were partially breastfed. Exclusive breastfeeding was uncommon, even in children less than six months old. Of note, seven case children who were exclusively breastfed had a *Cryptosporidium* infection, possibly indicating that a generally contaminated environment or the caretaker's hygiene practices may be contributing to *Cryptosporidium* transmission in the absence of transmission through water or food. Washing hands before nursing or preparing a baby's food was only reported by 28% of caretakers (see Table 9). Possible exposure routes for exclusively breastfed children may include person-to-person transmission or transmission via fomites, such as household objects or toys.

Drinking Water Source

Using rainwater as a main source of drinking water was common in our study population and was significantly negatively associated with *Cryptosporidium* infections in GEMS Kenya case children. The proportion of households using rainwater as a main source of drinking water varied greatly from month to month, ranging from less than 5% to more than 70%. Rainwater use often increased after the rainiest months of the year; however, rainwater use did not always increase or decrease with precipitation, likely due to the high proportion of households who report using stored drinking water. The proportion of households using surface water as a main source of drinking water (which was also common in our study population) decreased in the months that rainwater use increased. The WHO UNICEF Joint Monitoring Program classifies rainwater as an improved water source, whereas surface water is considered unimproved[74], showing that households may be relying more heavily on unimproved water sources when rainwater is not available. This underscores the importance of households having access to ample safe water storage containers for rainwater or having access to other improved sources of drinking water.

Water Treatment

Boiling water was significantly negatively associated with *Cryptosporidium* infections in GEMS case children. None of the other water treatment methods assessed was found to be associated with *Cryptosporidium* infection. Of the 1,476 case children, only one child came from a household that reported treating drinking water by filtration, and it was unclear the type of filtration used in this instance. As a consequence of the GEMS finding outlined here, and given the fact that filtration via ceramic water filters is thought to be an effective method of *Cryptosporidium* removal from drinking water, a small trial of the health impacts of ceramic water filters among households with infants is being carried out presently in the area where GEMS took place in Kenya.

Improved Sanitation

We were not able to assess having an improved sanitation facility in our multivariable models because so few (three) case children with *Cryptosporidium* had improved sanitation facilities; however, in unadjusted analysis, *Cryptosporidium* was associated with not having an improved sanitation facility. Improved sanitation infrastructure in rural Kenya would be a significant undertaking, but could possibly reduce childhood cryptosporidiosis in the region.

Cryptosporidium Genotypes

The majority of *Cryptosporidium* specimens from GEMS Kenya case children were of the genotype *C. hominis*, indicating that infections may more commonly result from exposure to human feces, rather than animal feces. This information may be important to the understanding of transmission routes for *Cryptosporidium* in this region.

Limitations

Associations between *Cryptosporidium* and environmental, behavioral, and clinical characteristics were assessed among GEMS Kenya case children with MSD. The potential risk factors for *Cryptosporidium* infection that were considered in screening may be similar to risk factors for MSD and *Cryptosporidium* itself is a risk factor for MSD, meaning that we conditioned on what is potentially a common effect of the exposures and outcome by making MSD a requirement for inclusion in this analysis. This is considered a type of structural selection bias and spurious associations can arise[79]. Considering GEMS case and control children together using sample weights (which have only recently become available) to reconstruct the source population would remove this limitation and will be investigated in future analyses (see "Recommendations and Future Research").

Several variables were dichotomized since attempting to include variables with more than two categories led to unstable multivariable regression models resulting from empty strata. Of note, main source of drinking water was originally collected as an 18-category variable and if included as such would have led to an unstable model. Therefore we screened on binary variables that compared each water source to all other sources combined. The dichotomization may limit the ability to interpret data. Sensitivity analyses may be able to address different categorization strategies in future research.

We were unable to assess breastfeeding and using milk as a home treatment for diarrhea in the two multivariable logistic regression models because of collinearity with age category. Additionally, inclusion of variables that occurred in fewer than five children with *Cryptosporidium,* such as having an improved sanitation facility, could not be assessed in the multivariable regression model because the model became unstable from the arising empty or sparse strata.

For the multivariable logistic model that specifically considered potential risk factors for *Cryptosporidium* infection, there may have been misclassification of exposures resulting from the questionnaire being administered at the time the child presented for care at the health facility, possibly leading to recall bias.

Follow-up information was only available at the 60-day follow-up home visit for children with MSD, limiting the ability to study longer-term outcomes of GEMS case children with *Cryptosporidium*.

Rainfall and temperature data were collected from a single weather station approximately 50-65km from the study site. A weather station was not available within the study area itself. The variables used for rainfall and temperature were the average millimeters of rainfall and average daily maximum temperature in the two weeks prior to enrollment; however, no sensitivity analysis was performed to see if this two-week lag best fit the data.

On collecting information on available sanitation facilities, the presence of a slab was not recorded for those who had pit latrines, leading to possible misclassification of traditional pit latrines as unimproved sanitation facilities, although the presence of slabs is thought to be low in the area.

Genotyping of *Cryptosporidium* was not available for all specimens.

Recommendations and Future Research

There is a need for a readily-available and reliable rapid diagnostic test for *Cryptosporidium* in rural western Kenya, which could be used for clinical decision making. Clinicians should provide education to caretakers on the importance of seeking a diagnosis for MSD and recognizing the signs of MSD, as well as early management and treatment of early childhood diarrheal episodes.

Nitazoxanide has been shown to decrease the duration of illness for immunocompetent individuals with *Cryptosporidium* infections, but is not often used in developing countries[6]. Increased availability of nitazoxanide and of rapid tests for *Cryptosporidium* could help reduce the burden of disease from cryptosporidiosis in rural Kenya. However, nitazoxanide is not indicated for use in children under one year old, so future research is needed for management of cryptosporidiosis in infants. Consistent use of antiretrovirals in the region may also decrease the severity of cryptosporidiosis among those with HIV or AIDS[41, 42].

There is no vaccine currently available for prevention of *Cryptosporidium* infections, although presumed acquired immunity to infection suggests that a vaccination could be an effective preventive measure[6]. Future research into a vaccine for *Cryptosporidium* is needed. In the absence of an effective vaccine, preventive measures may include sanitation infrastructure improvements and access to safe water storage containers for harvested rainwater or other provisions for increasing access to improved water sources, such as use of ceramic water filters or other effective point-of-use water treatment methods. Promotion of handwashing after defecating and at other key times may also be beneficial.

Follow-up of GEMS Kenya children with *Cryptosporidium* could provide valuable information on the longer-term effects of cryptosporidiosis in early childhood in Kenya and may yield results that would highlight the need to diagnose and treat *Cryptosporidium* infections.

Future planned analyses with currently-available data include: (1) studying risk factors of *Cryptosporidium* infections in both GEMS case and control children using sampling weights obtained from health utilization surveys to construct population estimates for the GEMS Kenya study region and (2) looking at children's individual changes in weight-for-age, height-for-age, and weight-for-height z-scores between enrollment and follow-up and comparing these between children with and without *Cryptosporidium*.

CONCLUSION

This study examined the outcomes and consequences of *Cryptosporidium* infections among GEMS Kenya case children with moderate-to-severe diarrhea and assessed environmental, behavioral, and clinical characteristics associated with *Cryptosporidium* infections in this population.

Overall, 10.8% of 1,476 case children had a *Cryptosporidium* infection, most of whom also had a co-infection with one or more additional enteric pathogens. Of the 152 children with a *Cryptosporidium* infection who had available follow-up information, 4.6% had died by the time of the 60-day follow-up.

Caretakers of children with *Cryptosporidium* infections reported similar clinical signs and symptoms during the children's diarrheal episodes as did caretakers of other case children with MSD who did not have a *Cryptosporidium* infection.

Of the case children who had a *Cryptosporidium* infection identified at baseline who had available 60-day follow-up information, the proportions who were underweight, stunted, at wasted were 17%, 26%, and 7% respectively at baseline and rose to 31%, 39%, and 15% by the time of the 60-day follow-up. Although there was no significant difference between the proportion of case children with and without *Cryptosporidium* infections who were underweight, stunted, or wasted at baseline, case children with *Cryptosporidium* infections were significantly more likely to be underweight or wasted by the time of the 60-day follow-up compared to children without a *Cryptosporidium* infection.

Characteristics significantly positively associated with *Cryptosporidium* infections included young age (less than two years), producing a watery stool sample at enrollment, and having flaky skin at enrollment. Characteristics significantly negatively associated with *Cryptosporidium* infections included having a rotavirus infection, coming from a household in which rainwater was the main source of drinking water, coming from a household that reports boiling drinking water, and coming from a household in which the caretaker reports washing hands after defecation.

Cryptosporidium contributes significantly to the burden of diarrheal illness in the study site, particularly among those less than two years old. Rapid and reliable diagnostic tests for *Cryptosporidium*, improved diarrhea case management and treatment of cryptosporidiosis, vaccine research, adequate access to improved drinking water, and future research on ceramic water filters are recommended for reducing the burden of cryptosporidiosis in rural western Kenya.

Additional Figures

Figure 4. Rainwater and Surface Water Use among GEMS Kenya Case Children, 2008

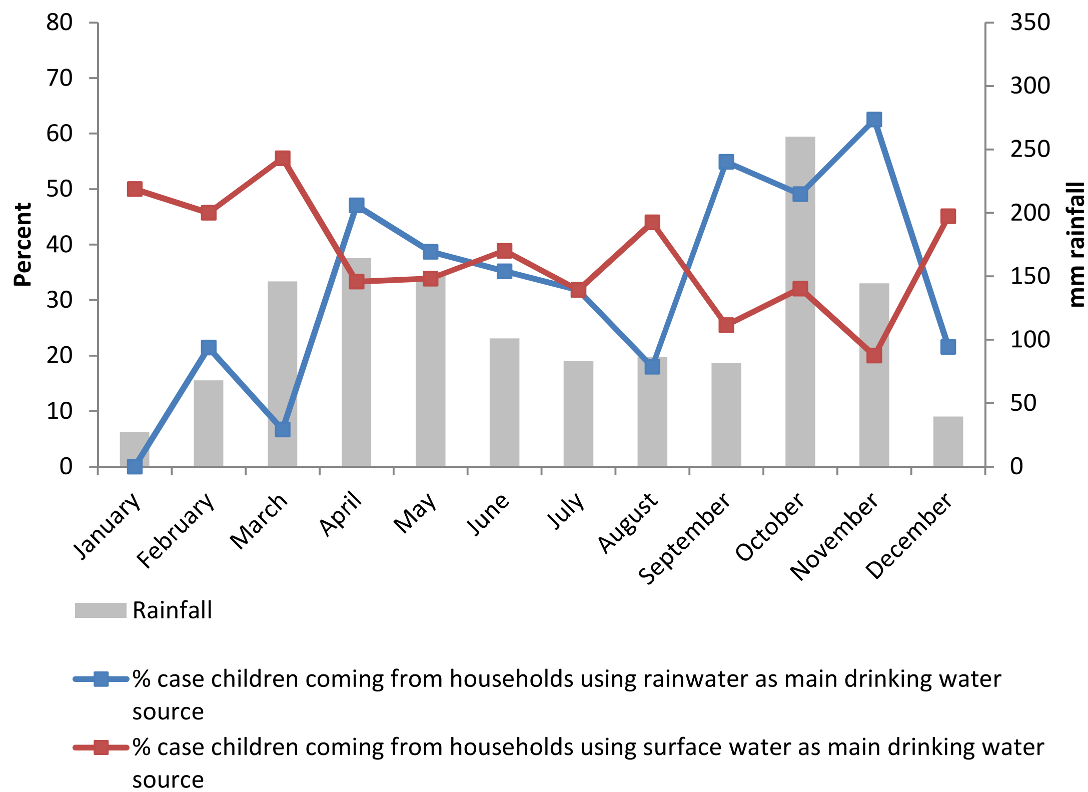


Figure 5. Rainwater and Surface Water Use among GEMS Kenya Case Children, 2009

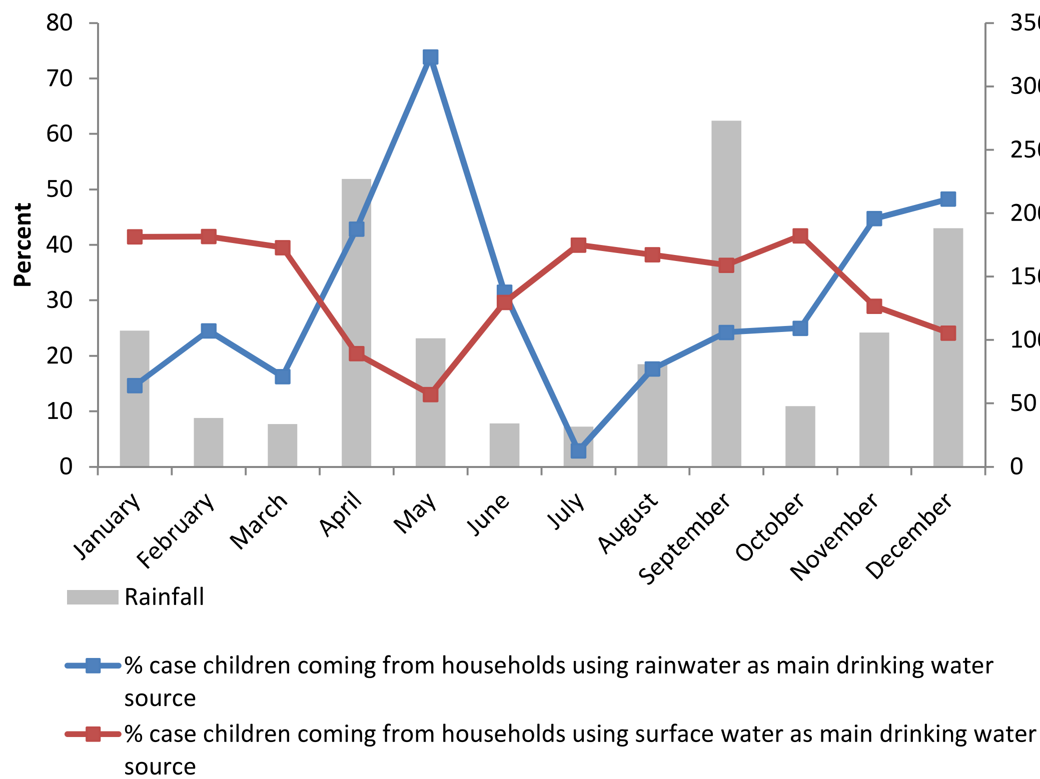
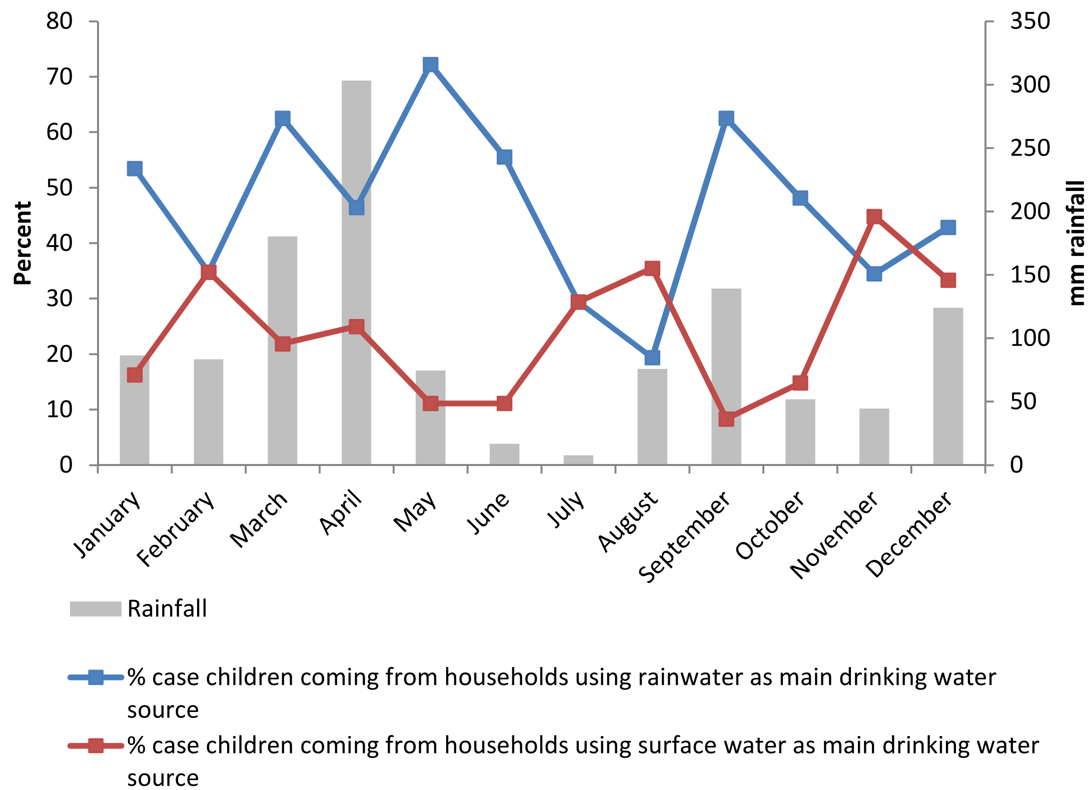


Figure 6. Rainwater and Surface Water Use among GEMS Kenya Case Children, 2010



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APPENDIX

Appendix 1. Variables Considered for Multivariable Logistic Regression Models

Variable	Collected	Screened/Coded
Household Characteristics		
Number of people living in household*	continuous	continuous
Number of people sleeping in household*	continuous	continuous
Number of children under 5 years in household*	continuous	continuous
Ownership of refrigerator*	binary	binary
Demographics		
Sex*	boy or girl	boy or girl
Age*	birth date and enrollment date recorded	categorized as: 0-11 months 12-23 months 24-59-months
Caretaker level of education*	no formal schooling, less than primary, completed primary, completed secondary, post-secondary, religious education only, or don't know	binary: caretaker completed at least primary school (yes/no)
Primary caretaker*	primary caretaker is: mother, father, sister, brother, grandmother, grandfather, aunt, uncle, not related, other	binary: primary caretaker is mother (yes/no)
Wealth index*	calculated: see definitions	quintiles
* variable considered for the risk factor model in addition to the exploratory model		

Appendix 1. Variables Considered for Multivariable Logistic Regression Models, continued

Health		
Breastfeeding*	none, partial, exclusive	0 = no, 1 = yes
Child HIV status*	binary (positive/negative)	binary (positive/negative)
Maternal HIV status*	binary (positive/negative)	binary (positive/negative)
Symptoms during diarrheal episode, as reported by caretaker at enrollment:		
Blood in stools	binary	binary
Vomiting 3 or more times per day	binary	binary
Very thirsty	binary	binary
Drank much less than usual	binary	binary
Unable to drink	binary	binary
Abdominal pain	binary	binary
Fever	binary	binary
Irritable or restless	binary	binary
Decreased activity or lethargy	binary	binary
Loss of consciousness	binary	binary
Rectal straining	binary	binary
Rectal prolapse	binary	binary
Cough	binary	binary
Difficulty breathing	binary	binary
Convulsion	binary	binary
Flaky skin observed at enrollment	binary	binary
Bipedal edema observed at enrollment	binary	binary
Abnormal hair observed at enrollment	binary	binary
Undernourishment observed at enrollment	binary	binary
* variable considered for the risk factor model in addition to the exploratory model		

Appendix 1. Variables Considered for Multivariable Logistic Regression Models, continued

Stool Characteristics (stool sample at enrollment)		
Mucus in stool	binary	binary
Blood in stool	binary	binary
Pus in stool	binary	binary
Stool sample consistency	categorical: formed, soft, thick liquid, opaque watery, or rice water-clear watery	watery stool: yes/no (where watery stool is "opaque watery" or "rice water-clear watery")
Home Treatments for Diarrhea		
Given the following home treatment before coming to the hospital:		
Oral rehydration solution	binary	binary
Homemade maize fluid	binary	binary
Special milk or infant formula	binary	binary
Home remedy or herbal medication	binary	binary
Zinc tablet or syrup	binary	binary
Other liquid	binary	binary
Antibiotics	binary	binary
Water Source, Storage, and Treatment		
Reported usual treatment of drinking water*	binary	binary
Treated water by boiling*	binary	binary
Used ceramic water filter*	binary	binary
Left water in sun to disinfect*	binary	binary
Gave child untreated water in last 2 weeks*	binary	binary
Gave child stored drinking water*	binary	binary
* variable considered for the risk factor model in addition to the exploratory model		

Appendix 1. Variables Considered for Multivariable Logistic Regression Models, continued

Water Source, Storage, and Treatment, continued		
Main source of drinking water over the two weeks prior to enrollment is:		
Piped into house*	During the last two weeks, the main source of drinking water for the household (piped into house, piped into yard, public tap, open well in house or yard, open public well, pond or lake, deep tube well, shallow tube well, covered well in house or yard, covered public well, protected spring, unprotected spring, river or stream, dam or earth pan, rainwater, bought, borehole, or other)	main source is piped into house (yes/no)
Piped into yard*		main source is piped into yard (yes/no)
Public tap*		main source is public tap (yes/no)
Open well in house or yard*		main source is open well in house or yard (yes/no)
Open public well*		main source is open public well (yes/no)
Pond or lake*		main source is pond or lake (yes/no)
Deep tube well*		main source is deep tube well (yes/no)
Shallow tube well*		main source is shallow tube well (yes/no)
Covered well in house or yard*		main source is covered well in house or yard (yes/no)
Covered public well*		main source is covered public well (yes/no)
Protected spring*		main source is protected spring (yes/no)
Unprotected spring*		main source is unprotected spring (yes/no)
River or stream*		main source is river or stream (yes/no)
Dam or earth pan*		main source is dam or earth pan (yes/no)
Rainwater*		main source is rainwater (yes/no)
Bought*		main source is bought (yes/no)
Borehole*		main source is borehole (yes/no)
Other*		main source is other (yes/no)
Surface water*	created from main source of drinking water; see definitions	main source is surface water (yes/no)
* variable considered for the risk factor model in addition to the exploratory model		

Appendix 1. Variables Considered for Multivariable Logistic Regression Models, continued

Sanitation		
Household has an improved sanitation facility*	facility for human waste disposal is: flush toilet, ventilated improved pit latrine, traditional pit toilet, ventilated improved pit with water seal, pour-flush toilet, no facility, other type of facility	improved sanitation facility (see definitions); binary
Hygiene		
Reported handwashing:		
Before eating*	binary	binary
Before cooking*	binary	binary
Before nursing/preparing baby's food*	binary	binary
After defecating*	binary	binary
After cleaning a child who defecated*	binary	binary
After handling domestic animals*	binary	binary
Number of key times handwashing reported*	sum of affirmative responses to above handwashing questions	continuous
Uses soap, ash, or clay for handwashing*	binary	binary
* variable considered for the risk factor model in addition to the exploratory model		

Appendix 1. Variables Considered for Multivariable Logistic Regression Models, continued

Animal Exposure		
House has agricultural land*	binary	binary
Cow in compound*	binary	binary
Goat in compound*	binary	binary
Sheep in compound*	binary	binary
Rodents in compound*	binary	binary
Fowl in compound*	binary	binary
Cat in compound*	binary	binary
Dog in compound*	binary	binary
Other animal in compound*	binary	binary
Cooking fuel used in household is animal dung*	binary	binary
Other Pathogens Identified in Stool		
Aeromonas	binary	binary
Adenovirus (type 40/41)	binary	binary
Adenovirus (not type 40/41)	binary	binary
Astrovirus	binary	binary
C. coli	binary	binary
C. jejuni	binary	binary
Enteroaggregative *E. coli*	binary	binary
Enterotoxigenic *E. coli* (LT)	binary	binary
Enterotoxigenic *E. coli* (ST)	binary	binary
E. histolytica	binary	binary
Giardia	binary	binary
Norovirus GI	binary	binary
Norovirus GII	binary	binary
Rotavirus	binary	binary
* variable considered for the risk factor model in addition to the exploratory model		

Appendix 1. Variables Considered for Multivariable Logistic Regression Models, continued

Other Pathogens Identified in Stool, continued		
Non-typhoidal *Salmonella*	binary	binary
Sapovirus	binary	binary
Shigella	binary	binary
V. cholera O1	binary	binary
Atypical enteropathogenic *E. coli*	binary	binary
Typical enteropathogenic *E. coli*	binary	binary
Weather		
Average mm of rainfall in two weeks before enrollment*	daily mm rainfall; average was calculated	quartiles
Average daily maximum temperature in two weeks before enrollment*	daily maximum temperature; average was calculated	continuous
* variable considered for the risk factor model in addition to the exploratory model		

Appendix 2. Memory Aid Form to Record the Presence or Absence of Diarrhea

Please complete this form every day for each of the next 14 days.

1. Each morning when you wake up, decide whether your child had diarrhea during the previous day. *Diarrhea* means that your child passed 3 or more loos or watery stools that were not normal for him or her on that day.
2. Go to the correct day. "o" means today. "o o" means tomorrow, and so on. A day begins when you wake up in the morning and ends when you wake up the next morning.
3. If your child had diarrhea that day, mark "X" in the dark box for that day [X]. If your child did <u>not</u> have diarrhea, mark "X" in the white box for that day [X]. **Each day, make only one "X".**
4. If you forget a few days, try to start again on the correct day.
5. Keep this form in a safe place. We will come to your house to collect it in 60 days.


		DIARRHEA	NORMAL
(1)	o (today)		
(2)	oo		
(3)	ooo		
(4)	oooo		
(5)	ooooo		
(6)	oooooo		
(7)	ooooooo		
(8)	oooooooo		
(9)	ooooooooo		
(10)	oooooooooo		
(11)	ooooooooooo		
(12)	oooooooooooo		
(13)	ooooooooooooo		
(14)	ooooooooooooo o		