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Risk Factors and Clinical Characteristics Associated with Enteropathogenic *Escherichia coli* Infection Among Children Less than Five Years Old with Moderate-to-Severe Diarrhea in Rural Western Kenya, 2008-2012

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Abstract

Risk Factors and Clinical Characteristics Associated with Enteropathogenic *Escherichia coli* Infection Among Children Less than Five Years Old with Moderate-to-Severe Diarrhea in Rural Western Kenya, 2008-2012

By Kirsten Fagerli

Background: Enteropathogenic *Escherichia coli* (EPEC) infection is a major cause of diarrhea and contributor to mortality in children <2 years old in developing countries. Limited data are available on risk factors for EPEC in children. The purpose of this study was to describe the prevalence of EPEC infections, assess the clinical characteristics of EPEC, and assess risk factors associated with EPEC among children <5 years old with moderate-to-severe diarrhea (MSD) enrolled in the Global Enteric Multicenter Study at the Kenyan study site.

Methods: MSD was defined as ≥3 loose stools in the previous 24 hours, with onset in the previous 7 days, and ≥1 of the following characteristics: loss of skin turgor, sunken eyes, dysentery, required IV rehydration, or hospitalization. Stool samples were tested at enrollment for presence of enteric pathogens. Demographic, clinical, anthropometric, and environmental data were collected at enrollment and at a ~60-day follow-up visit. Multivariable logistic regression was used to assess the risk factors and characteristics associated with typical EPEC and atypical EPEC. Multivariable linear regression was used to assess linear growth faltering.

Results: Of the 1778 cases enrolled in the study, 135 (7.6%) cases had typical EPEC, and 97 (5.5%) cases had atypical EPEC. 65% of typical EPEC, and 50% of atypical EPEC cases were infants (0-11 months old). 9.2% of typical EPEC cases, and 4.2% of atypical EPEC cases died prior to the 60-day follow-up visit. Clinical characteristics associated with typical EPEC included loss of skin turgor (adjusted odds ratio [aOR] 2.86, 95%CI: 1.08-4.82) and convulsions (aOR 2.95, 95%CI: 1.17-7.45). Infant cases with typical EPEC compared to those without were associated with linear growth faltering (p=0.002) between enrollment and follow-up. Clinical characteristics associated with atypical EPEC included difficulty breathing (aOR 3.35, 95%CI: 1.38-8.14) and coughing (aOR 1.93, 95%CI: 1.13-3.30). No environmental factors assessed were found to be associated with EPEC infection.

Discussion: Typical EPEC is a significant contributor to morbidity and mortality among infants with MSD in rural Kenya, while the pathogenicity of atypical EPEC remains unclear. Interventions aimed at reducing the burden of EPEC and its sequelae should be urgently investigated, prioritized, and implemented.

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Introduction

Background

Diarrheal illnesses remain a leading cause of childhood morbidity and mortality in children <5 years old in developing countries [1]. The Global Enteric Multicenter Study (GEMS) was a 4-year, prospective, matched case-control study conducted to examine moderate-to-severe diarrhea (MSD) in children 0-59 months old [2]. GEMS was initiated to estimate the population-based burden and etiology of children with MSD in censused populations in three countries in Asia and four countries in Africa. The findings from GEMS aim to guide development and implementation of enteric vaccines and other public health interventions that can diminish morbidity and mortality from diarrheal diseases [2].

MSD was defined as having three or more loose stools in the previous 24 hours, with onset in the previous 7 days, and having one or more of the following MSD characteristics: loss of skin turgor, sunken eyes, required intravenous fluid rehydration, dysentery (blood in stool), or required hospitalization. All cases were enrolled at health facilities between 2008 and 2012 [1]. Stool specimens were taken for all children at enrollment and tested for enteric bacterial, viral and parasitic pathogens. Clinical examinations, risk factor surveys, anthropometry, surveillance for deaths, and verbal autopsies were administered. Caretakers were also responsible for recording the presence of diarrhea for 14 days following the child's initial enrollment. A follow-up visit at the home of each enrolled child was carried out approximately 60 days after initial enrollment to assess health status, repeat anthropometry, and carry out environmental observations in the home [1].

Enteropathogenic *Escherichia coli* (EPEC), an enteric bacterium, is a strain of pathogenic *Escherichia coli* characterized by its ability to produce attaching and effacing (A/E) lesions [3]. This study focuses on both strains of enteropathogenic *Escherichia coli* (EPEC): typical enteropathogenic *Escherichia coli* (typical EPEC) and atypical enteropathogenic *Escherichia coli* (atypical EPEC). Among all study sites, typical EPEC was found to have the highest risk of death between enrollment and follow-up in infants 0-11 months old, after adjusting for other pathogens and study site [1]. In Kenya, typical EPEC was also found to have the fourth-highest attributable fraction in the 0-11 months age category, and the fifth-highest attributable fraction in the 12-23 month age category.

Significance

While significant strides have been made in identifying the pathogenesis of EPEC, a vaccine for EPEC is currently not available for roll-out. Furthermore, diarrhea attributed to EPEC tends to present with classic watery diarrhea symptoms and testing is expensive and technically complex. As a result, diagnosis of the pathogen is uncommon, and generic antibiotics are prescribed as treatment. However, most strains of EPEC are multi-drug resistant, rendering antibiotic treatment ineffective [4-5]. This is problematic as EPEC infections have been associated with excess mortality, infantile diarrhea, and prolonged diarrhea [3,5-9].

The purpose of this project was to examine the risk factors and characteristics associated with EPEC infection and associated death among children less than 5 years old with MSD. The study will accomplish this objective by focusing on 3 specific aims: 1) to describe the prevalence of typical EPEC and atypical EPEC infections in children less than five years old with MSD and enrolled in GEMS at the site in western Kenya, 2) to assess clinical characteristics and sequelae

of typical and atypical EPEC infections in children less than five years old with MSD and enrolled in GEMS at the study site in western Kenya, and 3) to assess behavioral and environmental risk factors associated with EPEC infection. These characteristics include water, sanitation, and hygiene habits, household characteristics, nutritional status, whether the child was breastfed, and other characteristics.

Literature Review

Enteropathogenic *Escherichia coli* (EPEC) Characteristics, Transmission, and Reservoirs

Escherichia coli is an enteric bacterium that is both an important member of the normal intestinal microflora of humans and a disease causing pathogen, dependent upon the strain. *E. coli* resides in the mucosal layer of the colon, and typically colonizes the gastrointestinal tract of infants within a few hours of birth [6]. While the majority of commensal *E. coli* strains are rarely harmful to humans, young children and immunocompromised individuals remain at risk for disease. Additionally, there are six pathotypes of *E. coli* that are also capable of causing disease in healthy individuals [6]. These pathogens include Shiga toxin–producing E. coli (STEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC).

EPEC are gram-negative, facultatively anaerobic, rod-shaped bacteria that are excreted through feces. These enteric bacteria are characterized by their ability to produce attaching and effacing (A/E) lesions that attach to the intestinal epithelial cells, causing cytoskeletal changes that create an accumulation of polymerized actin underneath the bacteria. This causes the intestinal microvilli to become effaced, lifting the bacteria like a platform [6].

The median infectious dose in healthy adult volunteers was found to be 10^8-10^{10} organisms, with an incubation period of approximately 9-12 hours [7]. As with other pathogenic *E. coli* strains, EPEC is transmitted through the fecal-oral route, and can be spread though contaminated water, weaning foods, and contaminated surfaces.

There are two strains of EPEC: typical EPEC and atypical EPEC. These two strains have different genetic characteristics, serotypes, and virulence properties [8]. The only currently known reservoirs for infection for typical EPEC are humans, whereas both humans and animals are considered reservoirs for infection for atypical EPEC [8,48]. However, asymptomatic adults and children are thought to be the major reservoir of infection for both strains, as 17-20% of infants under two years old have been found to shed EPEC in their stool [9]. Studies have also shown that symptomatic children can shed EPEC for up to two weeks after all symptoms have ceased [10].

Clinical Characteristics of EPEC Infection in Children Under 5 Years Old

EPEC primarily causes cases of acute, watery diarrhea, vomiting and a fever. These symptoms often cause dehydration, which can lead to death [7]. However, patients with atypical EPEC typically experience milder and non-dehydrating diarrhea, in comparison to typical EPEC. While a variety of antibiotics have been used to treat EPEC, most strains of EPEC are multi-drug resistant, rendering antibiotic treatments ineffective [4-5].

Age

One of the most notable features about EPEC is that the disease is primarily confined to children under two years old. This finding is consistent among various studies, and in both industrialized

and developing countries [3,11,13-19]. Typical EPEC is strongly associated with infantile diarrhea in children less than one year old [8]. This is a defining difference between the strains of EPEC, as a study in Norway found that atypical EPEC infections were not common in children younger than one year old [14]. While EPEC can be found in the stool of children over 2 years old, and adults, there is no apparent correlation with diarrheal illness. This is thought to be the result of a strengthening immune system, or a loss of receptors for specific adhesion [7].

Breastfeeding is thought to protective against developing severe forms of diarrhea. Two possible explanations for this are that 1) breastfed infants are less likely to consume contaminated drinking water or food, and 2) breastfeeding allows children to receive maternal antibodies in breast milk [8,44]. This is thought to provide some immunity from enteric pathogens [14]. As with many enteric diseases, breastfed infants are thought have a lower incidence of EPEC infection.

Malnutrition

EPEC infections have been previously linked in increased malabsorption rates, as a result of increased food intolerance to glucose and cow's milk [20]. The association between food intolerance and malabsorption increases in cases of prolonged or persistent diarrhea. This decreased uptake of nutrients in the intestinal tract may lead to stunting and/or being underweight. However, limited information is currently available about the longitudinal effects on case children's nutritional status after a diarrheal episode resulting from a typical EPEC or atypical EPEC infection.

HIV Status

There is little research in this area. However, one study conducted in the Homa Bay district of Kenya found that HIV-infected children with acute diarrhea were almost three times as likely to have a typical EPEC infection as HIV-uninfected children [21]. This association remained significant even after adjusting for duration of exclusive breastfeeding, current breastfeeding, stunting, and wasting. Studies in Brazil and Iran also found higher rates of EPEC infection among HIV and other immunocompromised patients; however, all three studies largely vary on the prevalence of EPEC infections, and the percentage of HIV-infected children with EPEC [21-23].

This trend also appears to hold true when a child's caretaker is infected with HIV [24]. One possible explanation for this finding is that the child has increased exposure to environmental factors, such as water and contaminated foods [24]. However, further research needs to be conducted to determine whether an association exists between such environmental exposures, HIV infection, and EPEC.

EPEC Infection and Childhood Mortality

As a result of the risk factors and clinical characteristics presented above, EPEC has also been associated with a high case-fatality rate. In Brazil, EPEC's case-fatality rate (7.1%) was found to be the highest of any pathogen in children with diarrhea [11]. Other studies have found similar results where children hospitalized with diarrhea are more likely to have an EPEC infection than another infection [15,20].

Known Risk Factors for EPEC in Developing Countries

EPEC outbreaks are typically tied to contaminated infant formula, weaning foods, and water [5]. Poor hygiene and handwashing practices can also contribute to outbreaks if young children are in close proximity to each other. Additionally, because the only known reservoir for typical EPEC is humans, poor water quality, and improper disposal of human waste is thought to be a major contributing factor to contracting the disease.

Studies in Brazil have shown that improved water quality, sanitation practices, and hand hygiene can significantly reduce the rate of children diagnosed with EPEC infections [25]. As a result, it is recommended that effective point-of-use water treatments, such as chlorine, water filtration, or boiling, should be used to kill any potential bacteria living in the water [26]. Covering buckets and using a tap to remove water can also prevent recontamination of the drinking water. Additionally, caretakers can prevent an EPEC infection by properly disposing of feces and practicing good handwashing behaviors, especially around infants.

Prevalence of Atypical EPEC and Typical EPEC in Industrialized and Developing Countries

EPEC is a major cause of diarrhea in children younger than two years old throughout the world. Even today, it is estimated that EPEC causes 5-10% of all diarrheal illnesses in infants in developing countries [25]. In the 1940s and 1950s, EPEC was considered a frequent cause of diarrheal outbreaks in infants in the United States, United Kingdoms, and other developed countries [7]. While EPEC outbreaks have been less frequent in developed nations, outbreaks are still know to occasionally occur in daycare centers and pediatric units. Whereas typical EPEC was considered a leading cause of diarrheal illness in children 50 years ago, it rarely is reported in developed nations today [8]. However, atypical EPEC still remains a prominent

cause of diarrhea, as evidenced by studies conducted in Norway, Poland, and Seattle, WA [12,27-28].

While the incidence of reported EPEC infections has significantly decreased in developed countries in the past 50 years, EPEC remains a prominent pathogen attributed to infantile diarrhea in developing countries. This is primarily a result of typical EPEC, as indicated by studies conducted in Bangladesh, Uruguay, and Brazil [17-18,25,29]. However, recent studies have shown that as Brazil has become more industrialized since the early 1990s, the incidence of reported typical EPEC infections has decreased, while reported atypical EPEC infections have increased [25]. This trend has also been noted in other industrializing countries, such as Mexico [19]. While it is unclear why atypical EPEC has emerged while typical EPEC has declined, it is hypothesized to be the result of improved sanitary conditions, water treatment options, and hospital sterilization methods [8].

EPEC in Kenya

Currently, 46% of people living in rural communities in Kenya have access to improved drinking water. Only 32% of the people living in the Nyanza Province have access to improved sanitation facilities, with 73% of children's (0-24 months) stool being properly disposed. Furthermore, only 4% of households indicate that they have a handwashing station in their home. Therefore, it is not surprising that the reported incidence of diarrhoea among children less than five years old is approximately 16% [26].

While many studies are conducted in Kenya each year, most studies focus on HIV, maternal and child healthcare, and malaria prevention. Few studies have explored the risk factors associated

with developing a typical or atypical EPEC infection. However, in recent years researchers have begun investing resources in studying how enteric pathogens have an effect on other diseases, such as HIV. For example, Pavlinac et al. conducted a study in western Kenya exploring the prevalence of various enteric pathogens in HIV-infected children with diarrhea compared to HIV-uninfected children with diarrhea. The study concluded that EPEC was a significant cause of diarrhea in HIV-infected children [21].

Current research in Kenya seeks to understand why many strains of EPEC are resistant to antibiotics [30]. These findings may aid in developing a vaccine or effective treatment option for EPEC in the event of an outbreak, and provides a promising direction for the future of research.

Diagnostic Techniques for Atypical EPEC and Typical EPEC

Until recently, EPEC was defined only by its O serogroups. However, as additional serotypes were found to be associated with infantile diarrhea, and better diagnostic methods became available, the definition was refined to O:H serotypes [7]. As a result, EPEC was redefined based on its ability to produce the A/E lesions, and that the pathogen was negative for Shiga toxin [8].

Today, genotypic tests using multiplex PCR are used to determine the strain of *E.* coli present in a stool sample [7]. The targets that determine the bacterium's classification of EPEC include intimin, or *eae* gene, outer membrane protein adhesion, and the EPEC plasmid-encoded bundle-forming pilus (bfpA) [31]. Strains positive for bfpA and *eae* are classified as typical EPEC. If the strains are positive for *eae*, but not bfpA, they are classified as atypical EPEC [31].

Methodology

The Global Enteric Multicenter Study (GEMS): Overview and Enrollment

The data from this study was collected as part of the GEMS-1 (2008-2011) and the GEMS-1A (2011-2012), collectively known as GEMS. This study was a case-control study that enrolled children less than five years old with moderate-to-severe diarrhea (MSD) in seven countries in sub-Saharan Africa and Asia. The study's rationale, design, clinical and microbiologic methods, and essential assumptions of GEMS have been previously described [2, 32-33]. The overarching goal of GEMS was to identify the etiologic agents associated with MSD in children under five years old, in an effort to develop vaccines, and other interventions aimed to decrease child morbidity and mortality resulting from diarrhea [32]. GEMS aimed to enroll 220 MSD cases per year in each of three age strata (0-11, 12-23, and 24-59 months old) from selected health facilities, as well as 1-3 matched community controls [2]. Control children were age- gender- and village of residence-matched to case children. Cases and controls provided clinical, epidemiologic, anthropometric, and environmental data at enrollment and during a follow-up visit approximately 60 days later. Stool specimens were also provided at enrollment for the identification and characterization of potential diarrheal pathogens [2]. The results of this paper will focus strictly on the data collected from the study site in Siaya County (formally known as Nyanza Province), Kenya.

In order for a child to be considered to a case, the child must have been 0-59 months old, presented with MSD, resided within the demographic surveillance system area of the study site, and presented to a health facility after the onset of MSD. MSD was defined as having three or more loose stools in the previous 24 hours, with onset in the previous seven days, and having one or more of the following MSD characteristics: loss of skin turgor, sunken eyes, required

intravenous fluid rehydration, dysentery (blood in stool), or required hospitalization [32]. Enrollment in this study was limited to 8-9 cases every two weeks per age stratum. Children with MSD were excluded from the study if they sought treatment at a selected health care facility, but had been previously enrolled in GEMS and their follow-up visit was still pending [2].

Stool samples were taken at enrollment from case children, and tested for a wide array of known enteric pathogens [31]. All stool samples were inspected for adequate temperature and volume (>3 mL) prior to testing [31]. All stool samples were processed and tested at the KEMRI/CDC enterics laboratory, Kisumu, Kenya, and results for EPEC specifically were verified at the Doherty Institute at the University of Melbourne [31].

A clinical examination by a licensed clinician or nurse was required at enrollment in order for a child to be considered as a case. During enrollment, a trained enumerator was also present to collect anthropometric measurements and administer a risk factor questionnaire. This questionnaire examined demographic factors, as well as water, sanitation, hygiene, and breastfeeding habits, among others. Caretakers of case children were also asked about the child's diarrhea, how many days it had persisted, and were given a Memory Aid to record the presence of diarrhea for the 14 days following enrollment [2]. This Memory Aid (sample shown in Appendix I) was designed as a simple tool for recording the presence of diarrhea, regardless of literacy level.

A follow-up visit was conducted at the case child's home approximately 60 days after enrollment. At follow-up, a trained enumerator administered a questionnaire about the child's health status and subsequent illnesses. Water, sanitation, and hygiene observations were also

noted at the home. Additionally, children were physically examined, and anthropometric measures were taken again. The Memory Aids were also reviewed with the caretaker and collected during this visit.

Study Site

The Kenya GEMS site was located in the rural, western side of the country, near Lake Victoria in the Siaya County (formally known as Nyanza Province), as shown in Figure 1. The site is part of the U.S. Centers for Disease Control and Prevention (CDC) and Kenya Medical Research Institute's (KEMRI) Health and Demographic Surveillance System (HDSS) study area [34]. This demographic surveillance area covers 217 villages spread over 500 km^2, with a population density of approximately 135,000 people [34]. The HDSS is responsible for collecting information on births, deaths, and migration patterns in the province three times per year.

In 2013, the mortality rate for children less than 5 years old in the Nyanza Province is 91 deaths per 1000 live births. The infant mortality rate for the Nyanza Province is 60 deaths per 1000 live births [35]. In the province, the leading causes of mortality for post-neonates are malaria, anemia, diarrhea/dehydration, and pneumonia [34].

Enteropathogenic *Escherichia coli* Identification

Stool samples were tested for all known infectious enteric pathogens. All strains of diarrheagenic *E. coli,* including EPEC, were identified using a multiplex polymerase chain reaction (PCR). The EPEC targets sought by the multiplex PCR reaction included the EPEC intimin, or *eae* gene, outer membrane protein adhesion, and the EPEC plasmid-encoded bundle-forming pilus (bfpA) [31]. Strains positive for bfpA and *eae* were classified as typical EPEC. If the

strains were positive for *eae*, but not bfpA, they were classified as atypical EPEC. Case children with a typical EPEC or atypical EPEC infection may have had more than one pathogen isolated in their stool samples. Details on the detection methods for all other enteric pathogens were previously described by Panchalingam et al. [31].

Anthropometry

Weight and height were recorded for every case at enrollment and at the 60-day follow-up visit. Weight and height were reported to the nearest 0.1 kilogram and nearest 0.1 centimeter, respectively. Digital scales for weight measurements were calibrated at a minimum of once per week. A Shorr board® was used for the measurement of length/height. Children under 24 months old or unable to stand were measured in the recumbent position. Children that were well enough to stand and at least 24 months old stood while their height was measured [2]. Case children that required rehydration at the health facility were weighed a second time after receiving fluids to account for dehydration. Additionally, children that were observed for more than four hours at the health facility were weighed again prior to discharge from the healthcare facility. The last weight measurement taken during the enrollment phase was considered the baseline measurement.

Weight-for-age, weight-for-height, and height-for-age z-scores were then calculated using a WHO SAS macro and the WHO Child Growth Standards for the reference population [36-37].

HIV

Due to ethical considerations, voluntary HIV testing and counseling, and linkage to existing HIV results for mothers, fathers, and case children enrolled in GEMS were only available during the

final two years of the study [2]. During GEMS, national guidelines were implemented for provider-initiated HIV testing and counseling (PITC), resulting in the Kenya site becoming one of two study sites where voluntary testing was conducted [2]. HIV status was determined by linking the GEMS data to data collected in the HDSS via the CDC Kenya Global AIDS Program. Data for case children were retrospectively linked to the Home-Based HIV Counseling and Testing (HBCT) Program, and was prospectively linked to PITC. HBCT involves a specially trained HIV counselor testing the child for HIV at home. As per the national algorithms, case children were tested for HIV during HBCT if the child's biological mother tested HIV positive, or the mother was deceased [38]. PITC HIV testing was offered to all children and their caretakers at GEMS sentinel health facilities, regardless of their presenting symptoms. Children older than 18 months had a rapid HIV antibody test conducted, and children less than 18 months old had a confirmatory PCR test [39]. All children and caretakers identified as HIV positive were referred to a HIV care and treatment program.

Breastfeeding

Questions pertaining to breastfeeding practices were collected differently between the GEMS-1 questionnaire and the GEMS-1A questionnaire. As a result, the data was not compatible to combining for four years, thus only data from the GEMS-1 were presented and analyzed.

Definitions

Breastfeeding: "Exclusive" breastfeeding refers to children only drinking breast milk, without any supplemental foods or liquids. "Partial" breastfeeding refers to children who are given supplemental foods or liquids in addition breast milk.

Underweight: Children with a weight-for-age (WAZ) z-score that was greater than two deviations

away from the mean. Children were considered severely underweight if their weight-for-age z-score was greater than three deviations away from the mean.

Stunted: Children with a height-for-age (HAZ) z-score was greater than two deviations away from the mean. Children were considered severely stunted if their height-for-age z-score was greater than three deviations away from the mean.

Wasted: Children with a weight-for-height (WHZ) z-score was greater than two deviations away from the mean. Children were considered severely wasted if their weight-for-height z-score was greater than three deviations away from the mean.

Wealth index quintile: Created using principle component analysis by classifying each household into one of five wealth index quintiles, representing the poorest to the wealthiest quintiles. Each category was created from a wealth index score that incorporates the number of rooms in a household for sleeping, whether the household has electricity, a television, scooter/motorcycle, radio, bicycle, car/truck, telephone, refrigerator, finished flooring (parquet or polished wood, vinyl or asphalt strips, ceramic tile, cement, or carpet), or boat with a motor [40,56].

Improved water source: Improved water sources include water that is piped into the household or yard, public taps, deep tube wells, covered wells in house or yard, covered public wells, protected springs, rainwater, and bore holes [41]. When the structure is used properly, it adequately protects water from outside contamination, particularly fecal matter, as a result of its construction.

Surface water: Drinking water coming from a river, pond, lake, stream, dam, or earth pan [41]. This is in accordance with the WHO UNICEF Joint Monitoring Program (JMP) standards except for the inclusion of earth pans, which is considered a water source specific to Kenya [41].

Effective water treatment method: The methods considered to effectively kill harmful pathogens

in the drinking water if used appropriately included solar treatment, chlorination, boiling, and filtering through ceramic or other such filter [42]. Because not all water treatment methods are effective against all pathogens, only the water treatment options shown to be effective against EPEC were included in this analysis.

Statistical Analysis

Data were analyzed in SAS 9.4 (SAS Institute, Inc., Cary, NC). Univariate and multivariate logistic regression was used to explore the risk factors for EPEC infection. Multivariable linear regression was used to assess linear growth faltering.

Three multivariable logistic regression models were assessed to examine, clinical characteristics at enrollment, clinical characteristics at follow-up, and potential environmental risk factors for typical and atypical EPEC. The models were fit for both typical EPEC and atypical EPEC separately.

Age was included in each model as it is considered a significant effect modifier for both typical and atypical EPEC [3,8,11-13]. Logistic regression using exact procedures was used to screen each variable for inclusion into the model. While feces disposal facility was observed during follow-up, for the purposes of this analysis, the facility will be analyzed with the environmental risk factors, as the likelihood is low that a facility was built between enrollment and follow-up surveys. For screening, those variables with a p-value <0.20 were considered for inclusion into the models [54]. Collinearity was then assessed for each model using conditional indexes [52-53]. Because all variables had a conditional index less than 30, it was assumed that there were no issues with collinearity in any of the models. All two-way interactions were then included in

the respective models, and used to assess if any interactions were significant. Interaction terms were not included if the likelihood ratio test chi-square p-value was greater than 0.10 [43]. Backward elimination was then used on each model to remove the remaining variables until only the variables with a p-value <0.05 remained.

Breastfeeding was not included in the models because only GEMS-1 data was included in analysis of breastfeeding for reasons mentioned earlier. Instead, we calculated the relative risk of EPEC infection by breastfeeding status stratified by age groups (0-5, 6-11, 12-23 months).

Anthropometry measurements were compared at baseline and follow-up to see if there was a difference in nutritional status between enrollment and follow-up. Only children with recorded measurements at both baseline and follow-up were included in analysis. Weight-for-age, weight-for-height, and height-for-age standardized z-scores were compared using McNemar's test for paired proportions using exact procedures. To further assess whether linear growth faltering was greater in MSD cases with EPEC compared to cases without EPEC, multivariable linear regression models were used and stratified by age group (0-11, 12-23, 24-59 months), and were adjusted by age, base height-for-age at enrollment, and duration until follow-up.

Ethical Review

Informed consent was obtained in Dholuo, the local dialect, from all caretakers of participating children prior to enrollment in the study. All study protocols, including participants' HIV status collected by the CDC Global AIDS Program and linked to the study, 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) at the University of Maryland, School

of Medicine, Baltimore, MD (UMD Protocol #H-28327). The IRB for the Centers for Disease Control and Prevention, Atlanta, GA deferred its review to the University of Maryland IRB (CDC Protocol #5038).

Results

In total, 33 variables were screened when analyzing clinical characteristics, 8 variables were screened when analyzing follow-up characteristics, and 15 variables were screened for when analyzing environmental risk factors for both typical and atypical EPEC. Variables considered for inclusion in the screening and models are listed in Tables 2, 5, and 7. The model with environmental factors was not assessed for atypical EPEC, as no environmental risk factors were found to be significant after an initial variable screening for inclusion in the model. The model analyzing follow-up sequelae was also not assessed for atypical EPEC because age was the only variable found to be significant after screening.

When analyzing the final multivariable model for typical EPEC, 8 variables were considered for clinical characteristics, 2 variables were considered for follow-up characteristics, and 6 variables were considered for environmental risk factors. When analyzing the final multivariable model for atypical EPEC, 7 variables and 2 interaction terms (difficulty breathing & cough, IV rehydration & loss of skin turgor) were considered for clinical characteristics, and no variables were considered for environmental risk factors or follow-up sequelae. Because GEMS enrolled children based on MSD episodes, it is possible that children were enrolled more than once. For this reason, cases are still referred to as “case children” as the dataset is episode based.

Of the 1778 cases enrolled at the GEMS site in western Kenya between January 31, 2008 and September 30, 2012, 226 (12.7%) children tested positive for EPEC infection. Of the 1778 cases enrolled GEMS, 1606 were first time enrollments, and 34 cases did not have HDSS identification information provided to determine if they were enrolled once or multiple times. The 226 cases of EPEC infection were further broken down into typical EPEC and atypical EPEC cases. In total, there were 135 (7.6%) typical EPEC infections, and 97 (5.5%) atypical EPEC infections, including 6 children with both typical and atypical EPEC infections (Figure 2). Enteric co-infections were common, with 717 (40.3%) case children having at least two pathogens identified in their stool. Due to the small sample size of EPEC cases with only one pathogen identified in the stool (50 cases of typical EPEC, 20 cases of atypical EPEC), cases with enteric co-infections were included in the analysis. As a result, the 6 children with both typical and atypical EPEC infections were included in both analyses.

Demographic and Household Characteristics

Demographics and household characteristics of EPEC cases by strain types are displayed in Table 1. In general, EPEC infection was significantly more common in children under 12 months compared to older age groups (unadjusted OR: 2.04, 95% CI: 1.4-2.97). Of those with a typical EPEC infection, 88 (65.2%) were under 12 months old. Among the identified cases of typical EPEC, 30 (22.2%) were children 12-23 months old, and 17 (12.6%) of cases were children 24-59 months old. Case children <12 months had significantly higher odds of typical EPEC infection compared to older age groups (unadjusted OR: 3.08, 95% CI: 1.81-5.25). Of those with an atypical EPEC infection, 48 (49.5%) of enrolled cases were under 12 months old. However, infant status was not associated with atypical EPEC infection (unadjusted OR: 1.22, 95% CI: 0.73-2.05). Among the identified cases of atypical EPEC, 27 (27.8%) were children 12-23 months old,

and 22 (22.7%) of cases were children 24-59 months old. Due to the known associations between EPEC and age, specifically in children less than one year old, age was assessed in each adjusted model.

Overall, 51 (37.9%) cases with a typical EPEC infection, and 39 (40.2%) of cases with an atypical EPEC infection were female. Only 103 (45.6%) children with an EPEC infection had a primary caretaker who completed their primary education. There was no significant difference between the median number of people sleeping in the household for those cases with a typical EPEC infection (4 people) or an atypical EPEC infection (5 people) and those without an EPEC infection (4 people). The median number of children <5 years old living in the household was 2 children for those with typical EPEC, atypical EPEC, and without an EPEC infection. Households were grouped into five quintiles, with each of the wealth quintile being equally represented. Most case children lived in households that owned agricultural land, and had animals living on the compound (Table 1).

Clinical Characteristics Presented in EPEC

The clinical presentation of GEMS cases with MSD at enrollment is presented in Table 2 by EPEC infection status. Being an infant <12 months old was a significant risk factor for typical EPEC, even after adjusting for other variables in the model (adjusted OR: 2.85, 95% CI: 1.66-4.90). Age was not considered a significant risk factor for atypical EPEC infection among any age group. Case children that required hospitalization upon enrollment did not have statistically significant odds of having a typical EPEC infection compared to the cases that did not require hospitalizations (unadjusted OR: 1.32, 95% CI: 0.83-2.10). The major symptoms for typical EPEC reported among case children included fever (77.0%), irritable/restlessness (73.3%), cough

(67.4%), and belly pain (65.9%). The major symptoms for atypical EPEC reported among case children included fever (80.4%), cough (70.1%), irritable/restlessness (69.1%), and belly pain (59.8%). Approximately 27 (20.0%) typical EPEC cases required intravenous rehydration therapy, as well as 23 (23.7%) of atypical EPEC cases. Both typical and atypical EPEC independently had higher odds of needing IV rehydration than for cases without EPEC (unadjusted OR 1.59, 95% CI: 1.02-2.48; unadjusted OR 2.00, 95% CI: 1.22-3.24, respectively). Children presenting with loss of skin turgor at enrollment, a symptom of dehydration, had higher odds of a typical EPEC infection than children that did not present a loss of skin turgor (adjusted OR: 2.05, 95% CI: 1.34-3.13). This association was not present in atypical EPEC cases. Additional indicators of dehydration that were assessed include bipedal edema, flaky skin, and sunken eyes. However, none of these indicators proved to have a significant association between MSD and typical or atypical EPEC infection. Furthermore, there was no association between MSD cases and EPEC infection regarding a child's thirst, or ability to drink at enrollment.

At enrollment, difficulty breathing was reported in approximately 19% of case children, and coughing was reported in approximately 61% of cases. Difficulty breathing was reported in 22.2% of typical EPEC cases, and 26.8% of atypical EPEC cases. In the adjusted model, cases reporting difficulty breathing were found to have higher odds of atypical EPEC compared to those who did not report difficulty breathing (adjusted OR: 2.79, 95% CI: 1.11-7.05). There were also higher odds in reported cases of coughing among children with MSD and atypical EPEC compared to those who did not report coughing (adjusted OR: 1.93, 95% CI: 1.13-3.30). Because coughing was reported in over 50% of all MSD cases, it is possible that some cases who reported

difficulty breathing also reported a cough, however this interaction was not found to be significant.

Less commonly reported symptoms for typical and atypical EPEC include loss of consciousness (typical EPEC: 11.1%; atypical EPEC: 4.1%), rectal straining (typical EPEC: 10.4%; atypical EPEC: 4.1%), rectal prolapse (typical EPEC: 2.2%; atypical EPEC 0.0%), and convulsions. While rare, convulsions were reported in 4.4% of children with a typical EPEC infection (adjusted OR: 2.79, 95% CI: 1.11-7.05). Only 1.8% of MSD cases without typical EPEC reported convulsions. Convulsions were only reported in 1.0% of atypical EPEC cases.

The median days of diarrhea at enrollment was three days for both typical and atypical EPEC. While the majority of cases reported passing less than seven stools within the 24 hours prior to enrollment, over a quarter of cases with typical EPEC (28.8%) and atypical EPEC (30.9%) reported more than seven stools. Furthermore, 6 (4.4%) of typical EPEC, and 4 (4.1%) of atypical EPEC cases reported the case child producing more than 10 stools in the 24 hours prior to enrollment. Stool samples were collected during enrollment in order to characterize the consistency of each case's MSD. Mucus was the most commonly reported stool characteristic, and was present in approximately 71.1% of typical EPEC and 75.3% of atypical EPEC positive cases. Watery diarrhea was the second most common feature, reported in approximately 59% of typical and atypical EPEC stool samples.

Breastfeeding

Reported breastfeeding practices for case children <24 months old for the GEMS-1 time period is displayed in Table 3. While approximately one in five case children less than 6 months old

were exclusively breastfed, of the children that tested positive for typical EPEC infection, only 10% were exclusively breastfed. The risk of having a typical EPEC infection for those exclusively breastfed was 58% lower than the risk for those not breastfed, although not statistically significant. After 5 months, exclusive breastfeeding rapidly declined to less than 2% for all case children, regardless of EPEC infection. Partial breastfeeding is the most common frequency of breastfeeding among all age classifications, however the risk of having a typical EPEC infection for those partially breastfed was not different than the risk of cases not breastfeed. After a case child reached 12 months, the percentage of children not drinking any breast milk increased to approximately 25% among all typical EPEC-positive, atypical EPEC-positive, and EPEC-negative categories. Overall, there was not found to be an increased risk of typical or atypical EPEC infection among any age group based on breastfeeding status.

HIV Status

Information pertaining to the children's HIV status was available for 58.8% of the GEMS Kenya cases. Of the children with a typical EPEC infection, 4.2% were HIV positive, and 2.0% of children with an atypical EPEC infection were HIV positive. The odds of having typical EPEC was 37% higher for HIV-positive children than for HIV-negative children, although not statistically significant (unadjusted OR: 1.37, 95% CI: 0.41-4.60). There was also no association found between HIV-positive children compared to HIV-negative children and atypical EPEC (unadjusted OR: 0.61, 95% CI: 0.08, 4.59). Of the 1194 mothers of case children with known HIV status, 30.1% of children with typical EPEC had mothers that were HIV-positive, and 14.3% of children with atypical EPEC had mothers that were HIV-positive (unadjusted OR: 1.42, 95% CI: 0.87-2.31; unadjusted OR: 0.52, 95% CI: 0.25-1.06 respectively). Of the 581 fathers of case children with known HIV status, there were higher odds of children with typical EPEC when their fathers were

HIV-positive fathers compared to children with HIV-negative fathers (unadjusted OR: 2.52, 95% CI: 1.39-4.59). This association did not hold true for cases of atypical EPEC (unadjusted OR: 1.02,95% CI: 0.43-2.42).

Enteric Co-infections

The majority of cases with a typical EPEC (63.0%) and atypical EPEC (79.4%) had at least one additional enteric pathogen identified in their stool. Among enrolled MSD cases without EPEC, 22.0% did not have any pathogens detected, 41.8% only had one pathogen identified, and 36.2% of cases had more than one pathogen detected. The most common pathogen found coinciding with both typical and atypical EPEC was *Giardia* (typical EPEC: 13.0%, atypical EPEC: 23.2% respectively), followed by rotavirus (typical EPEC: 12.2%; atypical EPEC: 15.8%), and *Cryptosporidium* (typical EPEC: 12.2%; atypical EPEC: 11.6%), Table 4. A full list the enteric pathogens identified among these groups is listed in Table 4.

60-Day Follow-up

Enrolled children in GEMS at the western Kenyan site were visited 60-90 days following enrollment, where a second questionnaire was administered to caretakers, anthropometric measure were taken, and environmental observations were made around the home. The results are provided in Table 5. Again, age was a significant risk factor for typical EPEC among children less than 1 year old after adjusting for other variables in the model (adjusted OR: 2.96, 95% CI: 1.73-5.06). Age was not considered a significant risk factor for atypical EPEC infection among any age group. There were significantly higher odds of death within the follow-up period among case children with typical EPEC infection compared to those without typical EPEC (adjusted OR: 2.87, 95% CI: 1.47-5.57). Of the five case children who died at the health facility

at which they were enrolled, two children were positive for typical EPEC (unadjusted OR: 8.22, 95% CI: 1.36-49.63).

Each case child's overall health was reported by the caretaker during the follow-up visit. Diarrhea was the most commonly reported sequelae (typical EPEC: 67.9%; atypical EPEC: 69.5%), followed by coughing (typical EPEC: 31.0%; atypical EPEC: 31.4%) and vomiting (typical EPEC: 13.8%; atypical EPEC: 15.7%). Due to the rarity of many diseases that were investigated, not all variables were assessed in this model.

Anthropometric Measurements as Indicators of Malnutrition

A comparison of the indicators of malnutrition at enrollment and follow-up are reported in Table 6. At both baseline and follow-up, stunting was the most common indicator of malnutrition. At the 60-day follow-up visit, there was a higher percentage of children who were stunted (typical EPEC: 34.5%, atypical EPEC: 36.3%), or severely stunted (typical EPEC: 15.1%, atypical EPEC: 17.6%), compared to baseline.

Stunting, wasting, and being underweight were then analyzed using McNemar's test for paired proportions using exact procedures to determine if there was a relationship between the anthropometric indicators of malnutrition at baseline and follow-up. Among the children that had typical EPEC, and had anthropometric measurements taken at baseline and follow-up, there was a significant decrease in the number of children who were underweight from enrollment to follow-up (p=0.002). There was also a significant decrease among children with typical EPEC and were considered wasted from enrollment to follow-up (p=0.02).

At follow-up, 15.1% of the case children with typical EPEC and had anthropometric measurements taken at both baseline and follow-up were severely stunted, compared to only 9.2% of children being severely stunted at enrollment (p=0.04). To further analyze the association between stunting and EPEC, linear growth faltering (i.e. the change in HAZ score between enrollment and follow-up, ΔHAZ) was stratified by age and assessed using multivariable linear regression. Infant cases with typical EPEC compared to those without were found to be associated with linear growth faltering (p=0.002) between enrollment and follow-up (Table 7). There were no significant associations between the indicators of malnutrition and presence of atypical EPEC in children with MSD.

Environmental Characteristics

Potential environmental risk factors for typical and atypical EPEC infection are detailed in Table 8. As noted in previous models, age was a significant risk factor for typical EPEC among children less than 1 year old after adjusting for other variables in the model (adjusted OR: 2.45, 95% CI: 1.23-4.85). Atypical EPEC was not assessed in this multivariable logistic regression model, as none of the variable listed below were significant after screening. When examining episodes of MSD at the GEMS Kenya study site, there did not appear to be a significant association between the child's primary source of drinking water and EPEC. In fact, none of the environmental factors assessed were found to be associated with typical or atypical EPEC infection (Table 8).

Approximately 64.4% of typical EPEC-positive respondents, and 65.0% of atypical EPEC-positive respondents reported treating the household's drinking water. Of the caretaker respondents who reported treating their drinking water, 60.5% of typical EPEC-positive cases used an effective water treatment method. The main methods reported for effectively treating drinking

water were chlorination and boiling. Similarly, of the respondents who reported treating their drinking water, 63.0% of atypical EPEC positive respondents reportedly used an effective water treatment method.

The most common type of feces disposal facility observed among GEMS Kenya case enrollees was a traditional pit toilet (observed in 66.7% of those with typical EPEC, 72.2% of households with atypical EPEC, and approximately 64% of those without EPEC). Feces was observed in the yard or around the defecation area in 33.3% of households with typical EPEC positive cases, and 41.2% of households with atypical EPEC positive cases.

Handwashing behaviors of the respondent, and commonly the primary caretaker, were also reported. The majority of respondents reported washing their hands after defecating (typical EPEC: 85.2%, atypical EPEC: 79.4%), and before eating (typical EPEC: 75.6%, atypical EPEC: 83.5%).

Animal ownership and economic status were also examined in the model to determine if there are any significant risk factors in addition to water, sanitation, and hygiene characteristics. Overall, each of the wealth quintiles was equally represented. Economic status was not included in the final model, as it was screened out, and found not to have a significant association in typical or atypical EPEC. Both households with cases of typical and atypical EPEC had a slightly lower percentage of ruminant animals in the household than those with without EPEC (Table 8).

Discussion

This study sought to establish risk factors and characteristics associated with EPEC infection in children with MSD less than five years old at the GEMS Kenya site. Overall, typical EPEC was found to be a significant cause of morbidity and mortality in children <5 years old with MSD, while atypical EPEC was not. The results of the study found that the risk of typical EPEC was highest among infants, with over 65% of all typical EPEC cases occurring in infants. Clinical and follow-up assessments showed potential consequences associated with typical EPEC. Only clinical assessments found any potential health outcomes associated with atypical EPEC. Our analysis of atypical EPEC did not find any long-term consequences or environmental risk factors associated with the infection.

Age

Consistent with previous studies, the prevalence of typical EPEC associated with MSD was greatest in children less than 1 year old, and decreased with increasing age [8]. The prevalence of atypical EPEC was not found to be associated with a specific age group among children less than 5 years old. This is consistent with a Norwegian study that found little association between age and atypical EPEC [14]. The reason for the differences between typical EPEC and atypical EPEC regarding age remains unclear. It is possible that in regions endemic for typical EPEC, such as Kenya, infants acquire immunity after initial or repeated exposure to the pathogens. This mechanism is currently thought to be the underlying basis for the relationship between age and diarrhea in ETEC-positive children in developing settings [45].

While other studies have found that exclusive breastfeeding is protective against typical EPEC infection, our study did not find breastfeeding to be protective against typical or atypical EPEC infection among any age group [14,44,46]. However, our ability to assess this was limited as there was very little exclusive breastfeeding overall at the rural Kenyan site.

Clinical Characteristics Associated with EPEC

Clinical symptoms in children with EPEC infection have been well established [5,7,47]. These symptoms include fever, belly pain, vomiting, watery diarrhea with mucus, and dehydration, consistent with the findings in this study for both typical and atypical EPEC-positive cases with MSD.

Respiratory problems, such as cough and difficulty breathing, were found to be significantly associated with atypical EPEC infection. As no additional clinical characteristics were associated with atypical EPEC infection, it is possible that this association is the result of a co-infection with a respiratory pathogen, although no respiratory testing was carried out to confirm or repute this.

Hospitalizations and Mortality in Children with EPEC

Dehydration is a major cause of hospitalizations and mortality in children with MSD. As with many enteric pathogens associated with watery diarrhea, indicators associated with dehydration, such as loss of skin turgor and requiring IV rehydration, were found to be independently associated with typical EPEC infection. This provides one potential explanation for the high proportion of hospitalizations and deaths associated with typical EPEC.

Almost one-fifth of all typical EPEC cases were hospitalized. While studies elsewhere have found a strong association between typical EPEC infection and hospitalization, this study did not [20]. Because our study exclusively enrolled children <5 years old with cases of MSD at health facilities, it is not surprising that the overall proportion of children, both with and without typical EPEC, hospitalized for MSD was high.

A child with a typical EPEC infection was nearly 3 times as likely to die between enrollment and follow-up, as a child without a typical EPEC infection. This is consistent with other studies that have found a strong association between typical EPEC and mortality [11,20]. The fact that typical EPEC has been associated with mortality in multiple studies highlights the need for early detection and appropriate treatment of typical EPEC in order to decrease the risk of death in infected children.

Studies in Norway and Australia have found that children with atypical EPEC typically experience milder, non-dehydrating symptoms than children with a typical EPEC infection [12-13]. This is consistent with the findings in this paper.

EPEC Prevalence and HIV Status

The prevalence of typical EPEC or atypical EPEC infection was not found to be independently associated with HIV status. This contrasts previous studies, including one conducted in Kenya from 2011-2013, which found that typical EPEC was significantly higher in HIV-positive children than other pathogens [21-23]. The variation in these findings may result from the small sample size of HIV positive children (approximately 4%) in the HDSS which is likely a consequence of

very active HIV care and treatment programs and Prevention of Mother to Child Transmission Programs relative to other areas and countries (personal communication, Ciara O'Reilly).

Interestingly, while the mother's HIV status did not have an effect on EPEC infection among MSD cases, the father being HIV-positive resulted in higher odds of typical EPEC. While little research has been conducted on how a father's HIV status affects EPEC, it is possible that in home transmission of typical EPEC is facilitated more readily in that context. As a result of living in a home with a HIV infected individual, the child may be more likely to have increased exposure to environmental factors or poor hygienic conditions [24].

Co-infection and EPEC

Previous GEMS analysis also found that typical EPEC was significantly associated with MSD during the first 2 years of a child's life [1]. This association was not established among MSD case with atypical EPEC infection. These findings should not be surprising as typical EPEC's ability to cause diarrhea is well established, whereas the pathogenicity of atypical EPEC is still unknown [8,46]. Furthermore, almost 80% of all atypical EPEC cases contained at least one additional pathogen. The findings of this study, combined with the lack of defined symptoms associated with atypical EPEC, may support the notion that MSD in children with atypical EPEC is a substantially less severe infection than typical EPEC.

Malnutrition Associated with EPEC

At enrollment, approximately one-third of typical EPEC-positive MSD cases were stunted or underweight, and almost 20% of cases were wasted. Additionally, cases with typical EPEC were more likely to be severely stunted when examining baseline to follow-up measures as compared

to cases without typical EPEC. These results concur with previous studies suggesting that EPEC is associated with severe stunting [11,20]. EPEC infections have been previously linked in increased malabsorption rates, as a result of increased food intolerance to glucose and cow's milk [20]. This decreased uptake of nutrients in the intestinal tract is one possible explanation as to why cases were more likely to be severely stunting between enrollment and follow-up. Atypical EPEC was not found to be associated with stunting, wasting, or being underweight. These findings suggest that future research should focus on children after a typical EPEC infection to ensure they are receiving sufficient nutritional care and rehabilitation when leaving the health facility.

Environmental Risk Factors Associated with EPEC

Water, sanitation, and hygiene characteristics were not found to be significantly associated with typical or atypical EPEC infection. A large proportion of children enrolled in GEMS Kenya used unimproved water sources as their primary source of drinking water. Approximately one-third of children with typical or atypical EPEC had feces observed on their compound. Additionally, less than half of caretaker respondents reported washing their hands with soap. While none of these associations were found to be significantly associated with EPEC infection, the importance of safe drinking water, sanitation, hygiene (WASH) practices for decreasing the incidence of diarrheal illness is well established [55]. It is likely that these results are reflective of the general association between enteric pathogens and poor WASH practices, rather than a lack of an association between EPEC and WASH practices.

The results of our study found a lower percentage of ruminant animals being present on the compound for cases of MSD with a typical EPEC infection, than those without typical EPEC. One

possible explanation for this finding is that because the only known reservoirs of infection for typical EPEC are currently humans, animals are independent of infection, unlike many other enteric pathogens examined [8]. Both humans and animals are considered reservoirs for infection for atypical EPEC [48]. However, not enough research has been carried out among animals to make any definitive observations at this point in time.

Limitations

There were several limitations to this study that have been cited elsewhere [2]. The results of this study may not be generalized to all children <5 years in Kenya, as it was conducted in a single rural site in western Kenya. Additionally, because all comparisons were made between GEMS cases, the factors associated with typical EPEC and atypical EPEC compared to other individuals with MSD may be different from the possible risk factors when compared to healthy controls.

Because there were few cases of typical or atypical EPEC with only one pathogen isolated in their stool, cases with enteric co-infection were included in the study to maintain an adequate sample size. As a result, our ability to explore the data on those with single-pathogen infections was limited. Furthermore, as many enteric pathogens present with similar symptoms, it may be difficult to tease out the effects of EPEC infection compared to other pathogens in co-infection cases.

Recall and reporting bias may have been influenced by social and cultural factors, where caretakers may have underreported potential risk factors, such as storing and drinking untreated water [50]. Furthermore, health conditions, other than diarrheal status, were not

reported or tested for at enrollment. As a result, clinical characteristics, such as respiratory status could not be assessed in detail. Misclassification of exposures may result from the questionnaire being administered at the time of enrollment, thus leading to recall bias.
Our ability to assess breastfeeding was limited because only GEMS-1 data was analyzed, and because of the collinearity between breastfeeding and age. Assessing EPEC infection among HIV-positive children was also limited due to the small sample size of HIV-positive children. Follow-up information was only available at the 60-day follow-up visit, limiting the ability to study intermittent and longer-term outcomes of GEMS Kenya cases of typical or atypical EPEC infections.

Conclusion

Overall, this study examined the risk factors and consequences of EPEC infections among GEMS Kenya cases children with MSD. Of the 1778 cases enrolled in the study, 135 (7.6%) cases had a typical EPEC infection, and 97 (5.5%) cases had an atypical EPEC infection. The risk of typical EPEC was highest among infants, with over 65% of all typical EPEC cases occurring in children in that age group. Of the 1717 cases with follow-up data available, 9.2% of children with typical EPEC, and 4.2% of children with atypical EPEC had died between enrollment and the 60-day follow-up visit.

Clinical characteristics found to be associated with typical EPEC included loss of skin turgor (an indicator of dehydration) and convulsions. Cases with typical EPEC were also more likely to be stunted at follow-up measures compared to MSD cases at enrollment. Clinical characteristics found to be associated with atypical EPEC include coughing and difficulty breathing.

It is estimated that EPEC causes 5-10% of all diarrheal illnesses in infants in developing countries [25]. Our study found typical EPEC to be a significant contributor to morbidity and mortality among infants with MSD in rural Kenya. Our found study did not find atypical EPEC to be a significant contributor to morbidity or mortality among the same study population. While a variety of antibiotics have been used to treat EPEC, most strains of EPEC are multi-drug resistant, rendering the treatments ineffective [4-5]. Additionally, poor water quality and improper disposal of human waste is thought to be a major contributing factor to contracting EPEC, as well as other enteric diseases. As a result, interventions aimed at reducing the burden of EPEC and its sequelae should be urgently investigated, prioritized, and implemented in order to improve diarrheal case management, vaccine research, and access to safe water, sanitation, and hygiene practices in rural western Kenya.

References

[1] Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., ... & Levine, M. M. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet*, *382*(9888), 209-222.

[2] Kotloff, K. L., Blackwelder, W. C., Nasrin, D., Nataro, J. P., Farag, T. H., van Eijk, A., ... & Levine, M. M. (2012). The Global Enteric Multicenter Study (GEMS) of diarrheal disease in infants and young children in developing countries: epidemiologic and clinical methods of the case/control study. *Clinical infectious diseases*, *55*(suppl 4), S232-S245.

[3] Ochoa, T. J., & Contreras, C. A. (2011). Enteropathogenic E. coli (EPEC) infection in children. *Current opinion in infectious diseases*, *24*(5), 478.

[4] Viscardi, M., Perugini, A. G., Auriemma, C., Capuano, F., Morabito, S., Kim, K. P., ... & Iovane, G. (2008). Isolation and characterisation of two novel coliphages with high potential to control antibiotic-resistant pathogenic Escherichia coli (EHEC and EPEC). *International journal of antimicrobial agents*, *31*(2), 152-157.

[5] Heyman DL, ed. *Control of communicable diseases manual*. 20th edn. Washington DC: American Public Health Association, 2014.

[6] Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic escherichia coli. *Nature Reviews Microbiology*, *2*(2), 123-140.

[7] Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic escherichia coli. *Clinical microbiology reviews*, *11*(1), 155-163.

[8] Trabulsi, L. R., Keller, R., & Tardelli Gomes, T. A. (2002). Typical and atypical enteropathogenic Escherichia coli. *Emerging infectious diseases*, *8*(5), 508-513.

[9] Levine M. M., Edelman R. (1984) Enteropathogenic Escherichia coli of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. Epidemiol. Rev. 6:31–51.

[10] Hill S. M., Phillips A. D., Walker-Smith J. A. (1991) Enteropathogenic Escherichia coli and life threatening chronic diarrhoea. Gut 32:154–158

[11] Fagundes-Neto, U., & Scaletsky, I. C. A. (2000). The gut at war: the consequences of enteropathogenic Escherichia coli infection as a factor of diarrhea and malnutrition. Sao Paulo Medical Journal, 118(1), 21-29.

[12] Afset, J. E., Bergh, K., & Bevanger, L. (2003). High prevalence of atypical enteropathogenic Escherichia coli (EPEC) in Norwegian children with diarrhoea. Journal of medical microbiology, 52(11), 1015-1019.

[13] Nguyen, R. N., Taylor, L. S., Tauschek, M., & Robins-Browne, R. M. (2006). Atypical enteropathogenic Escherichia coli infection and prolonged diarrhea in children. Emerging infectious diseases, 12(4), 597.

[14] Afset, J. E., Bevanger, L., Romundstad, P., & Bergh, K. (2004). Association of atypical enteropathogenic Escherichia coli (EPEC) with prolonged diarrhoea. Journal of medical microbiology, 53(11), 1137-1144.

[15] Guerrant, R. L., Kirchhoff, L. V., Shields, D. S., Nations, M. K., Leslie, J., De Sousa, M. A., ... & Hughes, J. M. (1983). Prospective study of diarrheal illnesses in northeastern Brazil: patterns of disease, nutritional impact, etiologies, and risk factors. *Journal of infectious diseases*, *148*(6), 986-997.

[16] Trabulsi LR, Keller R, Tardelli Gomes TA. (2002) Typical and atypical enteropathogenic *Escherichia coli*. Emerg Infect Dis. 8(5):508-13.

[17] Blanco, M., Blanco, J. E., Dahbi, G., Mora, A., Alonso, M. P., Varela, G., ... & Blanco, J. (2006). Typing of intimin (eae) genes from enteropathogenic Escherichia coli (EPEC) isolated from children with diarrhoea in Montevideo, Uruguay: identification of two novel intimin variants (μB and ξR/β2B). Journal of medical microbiology, 55(9), 1165-1174.

[18] Gomes TAT, Vieira MA, Wachsmuth IK, Blake PA, Trabulsi LR. Serotype-specific prevalence of Escherichia coli strains with EPEC adherence factor genes in infants with and without diarrhea in São Paulo, Brazil. J Infect Dis 1989;160:131-5.

[19] Estrada-Garcia, T., Lopez-Saucedo, C., Thompson-Bonilla, R., Abonce, M., Lopez-Hernandez, D., Santos, J. I., ... & Long, K. Z. (2009). Association of diarrheagenic Escherichia coli pathotypes with infection and diarrhea among Mexican children and association of atypical enteropathogenic E. coli with acute diarrhea. Journal of clinical microbiology, 47(1), 93-98.

[20] Fagundes-Neto, U., & Andrade, J. A. D. (1999). Acute diarrhea and malnutrition: lethality risk in hospitalized infants. Journal of the American College of Nutrition, 18(4), 303-308.

[21] Pavlinac, P. B., John-Stewart, G. C., Naulikha, J. M., Onchiri, F. M., Denno, D. M., Odundo, E. A., ... & Walson, J. L. (2014). High-Risk Enteric Pathogens Associated with HIV-Infection and HIV-Exposure in Kenyan Children with Acute Diarrhea. AIDS (London, England), 28(15), 2287.

[22] Alizade, H., Sharifi, H., Naderi, Z., Ghanbarpour, R., Bamorovat, M., & Aflatoonian, M. R. (2015). High Frequency of Diarrheagenic Escherichia coli in HIV-Infected Patients and Patients with Thalassemia in Kerman, Iran. Journal of the International Association of Providers of AIDS Care (JIAPAC), 2325957415617831.

[23] Rossit, A. R. B., de Almeida, M. T. G., Nogueira, C. A. M., da Costa Oliveira, J. G., Barbosa, D.

M. U., Moscardini, A. C., ... & Cavasini, C. E. (2007). Bacterial, yeast, parasitic, and viral enteropathogens in HIV-infected children from São Paulo State, Southeastern Brazil. Diagnostic microbiology and infectious disease, 57(1), 59-66.

[24] Arvelo, W., Kim, A., Creek, T., Legwaila, K., Puhr, N., Johnston, S., ... & Bowen, A. (2010). Case–control study to determine risk factors for diarrhea among children during a large outbreak in a country with a high prevalence of HIV infection. International Journal of Infectious Diseases, 14(11), e1002-e1007.

[25] Torres, A. G. (Ed.). (2010). Pathogenic Escherichia coli in Latin America. Bentham Science Publishers.

[26] UNICEF. (2011). Kenya, Nyanza Province: monitoring situation of children and women. Kenya National Bureau of Statistics.

[27] Bokete, T. N., Whittam, T. S., Wilson, R. A., Clausen, C. R., O'Callahan, C. M., Moseley, S. L., ... & Tarr, P. I. (1997). Genetic and phenotypic analysis of Escherichia coli with enteropathogenic characteristics isolated from Seattle children. Journal of Infectious Diseases, 175(6), 1382-1389.

[28] Paciorek, J. (2002). Virulence properties of Escherichia coli faecal strains isolated in Poland from healthy children and strains belonging to serogroups O18, O26, O44, O86, O126 and O127 isolated from children with diarrhoea. Journal of medical microbiology, 51(7), 548-571.

[29] Albert, M. J., Faruque, S. M., Faruque, A. S., Neogi, P. K., Ansaruzzaman, M., Bhuiyan, N. A., ... & Akbar, M. S. (1995). Controlled study of Escherichia coli diarrheal infections in Bangladeshi children. Journal of Clinical Microbiology, 33(4), 973-977.

[30] Bii, C. C., Taguchi, H., Ouko, T. T., Muita, L. W., Wamae, N., & Kamiya, S. (2005). Detection of virulence-related genes by multiplex PCR in multidrug-resistant diarrhoeagenic Escherichia coli isolates from Kenya and Japan. Epidemiology and infection, 133(04), 627-633.

[31] Panchalingam, S., Antonio, M., Hossain, A., Mandomando, I., Ochieng, B., Oundo, J., . . . Nataro, J. P. (2012). Diagnostic microbiologic methods in the GEMS-1 case/control study. *Clin Infect Dis, 55 Suppl 4*, S294-302. doi:10.1093/cid/cis754

[32] Levine, M. M., Kotloff, K. L., Nataro, J. P., & Muhsen, K. (2012). The Global Enteric Multicenter Study (GEMS): impetus, rationale, and genesis. *Clinical infectious diseases*, *55*(suppl 4), S215-S224.

[33] Farag, T. H., Nasrin, D., Wu, Y., Muhsen, K., Blackwelder, W. C., Sommerfelt, H., ... & Levine, M. M. (2012). Some epidemiologic, clinical, microbiologic, and organizational assumptions that influenced the design and performance of the Global Enteric Multicenter Study (GEMS). *Clinical infectious diseases*, *55*(suppl 4), S225-S231.

[34] Adazu, K., Lindblade, K. A., Rosen, D. H., Odhiambo, F., Ofware, P., Kwach, J., ... & Vulule, J. M. (2005). Health and demographic surveillance in rural western Kenya: a platform for evaluating interventions to reduce morbidity and mortality from infectious diseases. *The American Journal of Tropical Medicine and Hygiene*, *73*(6), 1151-1158.

[35] Bay, H. (2013). Kenya, Nyanza Province.

[36] World Health Organization. WHO child growth standards, SAS macros. Geneva, World Health Organization; 2010.

[37] De Onis M. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: World Health Organization; 2006.

[38] Wachira, J., Kimaiyo, S., Ndege, S., Mamlin, J., & Braitstein, P. (2012). What is the impact of home-based HIV counseling and testing on the clinical status of newly enrolled adults in a large HIV care program in Western Kenya?. *Clinical Infectious Diseases*, *54*(2), 275-281.

[39] O'Reilly, C., Rajasingham, A., Omore, R., Moke, F., Ondeng', A., Hukumu, E., Soo, L., Ochieng, B., Farag, T., Nasrin, D., Panchalingam, S., Nataro, J., Kotloff, K., Levine, M., Oundo, J., Parsons, M., Bopp, C., Vulule, J., Laserson, K., Mintz, E., & Breiman R. *HIV Infection Modifies Susceptibility to Certain Diarrheal Pathogens and Influences the Outcome of Moderate and Severe Diarrhea*. GEMS Symposium, ASTMH Annual Meeting. November 13, 2012. Atlanta, GA.

[40] Filmer D, Pritchett LH. Estimating wealth effects without expenditure data - Or tears: An application to educational enrollments in states of India. Demography 2001; 38(1): 115-32.

[41] WHO / UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation. Definitions and Methods: Introduction and Wat/San Categories. 2010. http://www.wssinfo.org/definitions-methods/watsan-categories/

[42] WHO. Results of Round I of the WHO International Scheme to Evaluate Household Water Treatment Technologies. 2016. http://www.who.int/water_sanitation_health/hygiene/om/linkingchap6.pdf

[43] Hegewald, J., Pfahlberg, A., & Uter, W. (2003, September). A Backwards-Manual Selection Macro for Binary Logistic Regression in the SAS® v. 8.02 PROC LOGISTIC Procedure. In *Proceedings of the North East SAS Users Group*.

[44] Levine MM, Robins-Browne RM. Factors that explain excretion of enteric pathogens by persons without diarrhea. *Clin Infect Dis* 2012;55(suppl 4):S303-11.

[45] Beutin, L. (2006). Emerging enterohaemorrhagic Escherichia coli, causes and effects of the rise of a human pathogen. *Journal of Veterinary Medicine, Series B*, *53*(7), 299-305.

[46] Okeke, I. N. (2009). Diarrheagenic Escherichia coli in sub-Saharan Africa: status, uncertainties and necessities. *The Journal of Infection in Developing Countries*, *3*(11), 817-842.

[47] Gomes, T. A. T., Rassi, V., MacDonald, K. L., Ramos, S. R. S., Trabulsi, L. R., Vieira, M. A., ... &

Blake, P. A. (1991). Enteropathogens associated with acute diarrheal disease in urban infants in São Paulo, Brazil. *Journal of Infectious Diseases*, *164*(2), 331-337.

[48] Hernandes, R. T., Elias, W. P., Vieira, M. A., & Gomes, T. A. (2009). An overview of atypical enteropathogenic Escherichia coli. *FEMS Microbiology Letters*, *297*(2), 137-149.

[49] Hu, J., & Torres, A. G. (2015). Enteropathogenic Escherichia coli: foe or innocent bystander?. *Clinical Microbiology and Infection*, *21*(8), 729-734.

[50] Manesh, A. O., Sheldon, T. A., Pickett, K. E., & Carr-Hill, R. (2008). Accuracy of child morbidity data in demographic and health surveys. *International Journal of Epidemiology*, *37*(1), 194-200.

[51] Maponga, B. A., Chirundu, D., Gombe, N. T., Tshimanga, M., Shambira, G., & Takundwa, L. (2013). Risk factors for contracting watery diarrhoea in Kadoma City, Zimbabwe, 2011: a case control study. *BMC infectious Diseases*, *13*(1), 1.

[52] Wax Y. Collinearity diagnosis for a relative risk regression analysis: an application to assessment of diet-cancer relationship in epidemiological studies. *Stat Med* 1992;11(10)**:**1273-87.

[53] Zejda JE. Collinearity in multivariable analysis: causes, detection and control measures. *Przegl Epidemiol* 2009;63(2)**:**333-7.

[54] Barros, A. J., & Hirakata, V. N. (2003). Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC medical research methodology*, *3*(1), 1.

[55] Clasen, T., Schmidt, W. P., Rabie, T., Roberts, I., & Cairncross, S. (2007). Interventions to improve water quality for preventing diarrhoea: systematic review and meta-analysis. *Bmj*, *334*(7597), 782.

[56] McKenzie DJ. Measuring Inequality with Asset indicators. Journal of Population Economics. 2005;18(2):229-60.

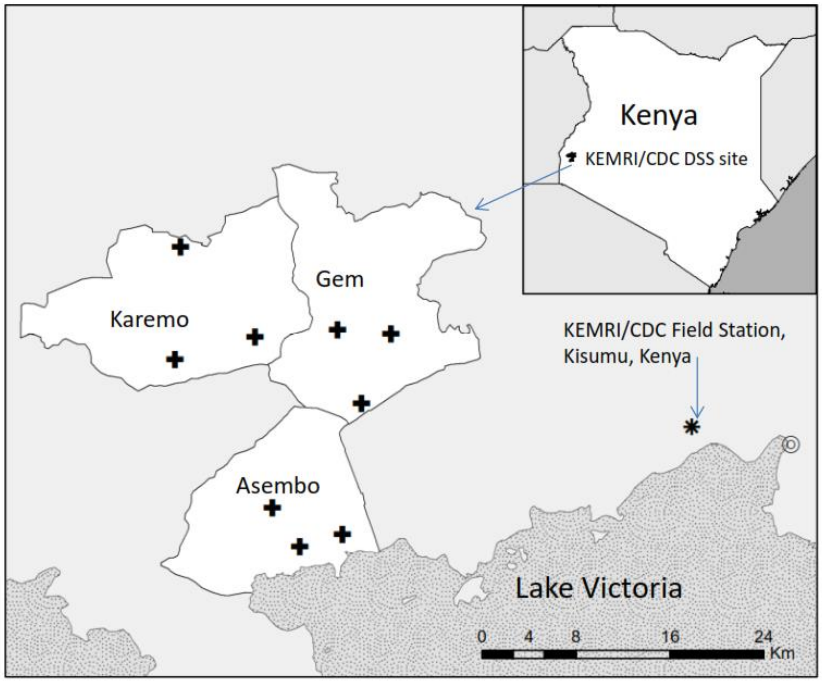

Figure 1. KEMRI/CDC HDSS study area (Asembo, Gem, and Karemo) where GEMS Kenya Study was conducted

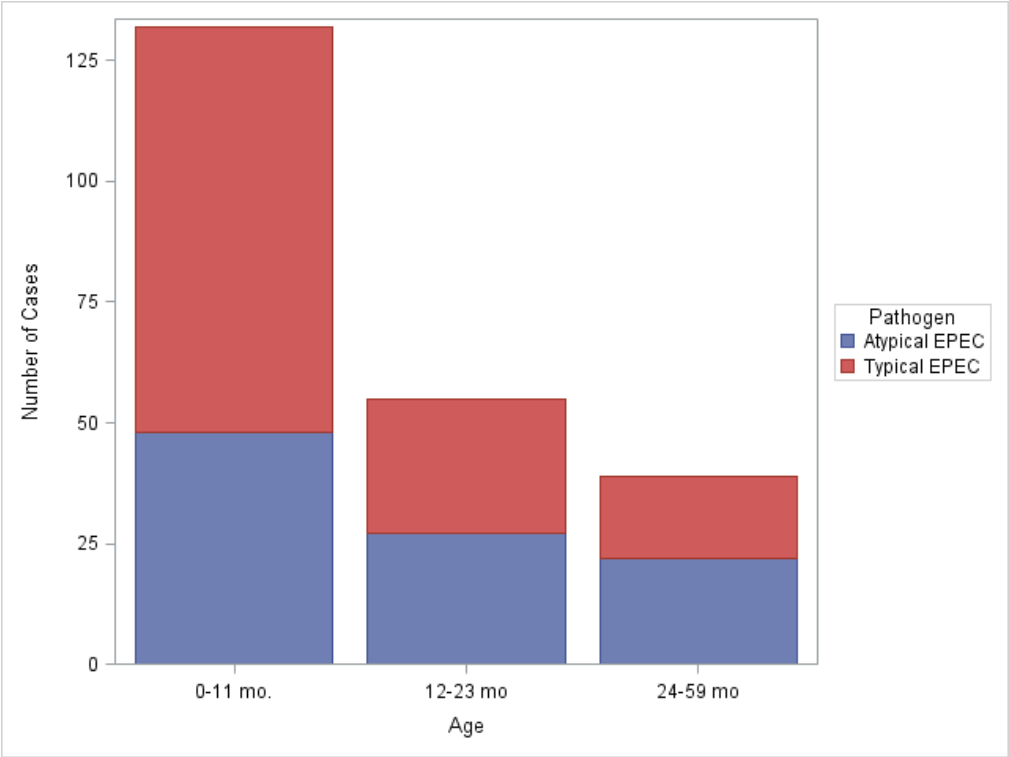

Figure 2. Number of cases of typical and atypical EPEC Infection among children <5 years old with MSD enrolled in GEMS by age group, rural western Kenya, 2008-2012

Table 1. Demographic and household characteristics of children with typical EPEC and atypical EPEC MSD (n=1778), rural western Kenya, 2008- 2012

		Typical EPEC		Atypical EPEC	
	Total (N=1778)	Positive (N = 135)	Negative (N = 1643)	Positive (N = 97)	Negative (N = 1681)
Age					
0-11 months	829 (46.6%)	88 (65.2%)	741 (45.1%)	48 (49.5%)	781 (46.5%)
12-23 months	491 (27.6%)	30 (22.2%)	461 (28.1%)	27 (27.8%)	464 (27.6%)
24-59 months	458 (25.8%)	17 (12.6%)	441 (26.8%)	22 (22.7%)	436 (25.9%)
Gender					
Female	768 (43.2%)	51 (37.9%)	717 (43.6%)	39 (40.2%)	729 (43.4%)
Primary Caretaker					
Mother	1714 (96.4%)	132 (97.8%)	1582 (96.3%)	89 (91.8%)	1625 (96.7%)
Completed primary school	796 (44.8%)	58 (43.0%)	738 (44.9%)	48 (49.5%)	748 (44.5%)
Household Characteristics					
People sleeping in house(median)	-	4	4	5	4
Above median	1108 (62.3%)	76 (56.3%)	1032 (62.8%)	69 (71.1%)	1039 (61.8%)
Young children in house (median)	-	2	2	2	2
Above median	203 (11.4%)	9 (6.7%)	194 (11.8%)	19 (19.6%)	184 (11.0%)
Owns agricultural land	1611 (90.6%)	124 (91.9%)	1487 (90.5%)	88 (90.7%)	1523 (90.6%)
Animals present in compound	1767 (99.4%)	132 (97.8%)	1635 (99.5%)	95 (97.9%)	1672 (99.5%)
Ruminant animal ownership	1375 (77.3%)	94 (69.6%)	1281 (80.0%)	71 (73.2%)	1304 (77.6%)
Wealth Index Quintile					
First quintile (poorest)	312 (17.6%)	21 (15.6%)	291 (17.7%)	11 (11.3%)	301 (17.9%)
Second quintile	366 (20.6%)	31 (23.0%)	335 (20.4%)	27 (27.8%)	339 (20.2%)
Third quintile	465 (26.2%)	31 (23.0%)	434 (26.4%)	19 (19.6%)	446 (26.5%)
Fourth quintile	291 (16.4%)	26 (19.3%)	265 (16.1%)	20 (20.6%)	271 (16.1%)
Fifth quintile (wealthiest)	344 (19.4%)	26 (19.3%)	318 (19.4%)	20 (20.6%)	324 (19.3%)

Table 2. Presentation of clinical symptoms in children with MSD (n=1778) by EPEC status at enrollment, rural western Kenya, 2008-2012

	Typical EPEC				Atypical EPEC			
	Positive (N = 135)	Negative (N = 1643)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Positive (N = 97)	Negative (N = 1681)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Gender								
Female	51 (37.9%)	717 (43.6%)	0.78 (0.55, 1.13)		39 (40.2%)	729 (43.4%)	0.88 (0.58, 1.33)	
Measured or observed by clinician at enrollment								
Child hospitalized	24 (17.8%)	231 (14.1%)	1.32 (0.83, 2.10)		16 (16.5%)	239 (14.2%)	1.19 (0.69, 2.07)	
IV rehydration	27 (20.0%)	223 (13.6%)	1.59 (1.02-2.48)	0.97 (0.59,1.61)	23 (23.7%)	227 (13.5%)	2.00 (1.22, 3.24)	0.95 (0.33-2.76)
Bipedal edema	2 (1.5%)	20 (1.2%)	1.22 (0.28,5.28)		2 (2.1%)	20 (1.2%)	1.75 (0.40, 7.59)	
Flaky skin	6 (4.4%)	43 (2.6%)	1.73 (0.72,4.14)		1 (1.0%)	48 (2.9%)	0.35 (0.05, 2.60)	
Loss of skin turgor	54 (40.0%)	352 (21.4%)	2.45 (1.70,3.52)	2.05 (1.34,3.13)	28 (28.9%)	378 (22.5%)	1.40 (0.89, 2.20)	0.60 (0.29,1.25)
Sunken eyes	127 (94.1%)	1538 (93.6%)	1.08 (0.52, 2.27)		92 (94.6%)	1573 (93.6%)	1.26 (0.50, 3.17)	
Under nutrition	19 (14.1%)	145 (8.8%)	1.70 (1.01,2.83)	1.02 (0.43,2.43)	6 (6.2%)	158 (9.4%)	0.64 (0.27, 1.47)	
Abnormal hair	12 (8.9%)	73 (4.4%)	2.10 (1.11,3.97)	1.64 (0.69,3.94)	4 (4.1%)	82 (4.8%)	0.85 (0.30, 2.37)	
Fever†	104 (77.0%)	1262 (76.8%)	1.01 (0.67, 1.54)		78 (80.4%)	1288 (76.6%)	1.25 (0.75, 2.09)	
Health status at enrollment*								
Child is thirsty	113 (83.7%)	1378 (83.9%)	1.00 (0.62, 1.62)		84 (86.6%)	1407 (83.7%)	1.32 (0.71, 2.45)	
Difficulty drinking/ unable to drink	8 (5.9%)	56 (3.4%)	1.79 (0.83, 3.83)		6 (6.2%)	58 (3.5%)	1.85 (0.78, 4.39)	
Offered less to drink than normal	74 (54.8%)	752 (45.8%)	1.44 (1.01,2.04)	1.14 (0.79-1.64)	39 (40.2%)	787 (46.8%)	0.76 (0.50,1.16)	
Offered less to eat than normal	108 (80.0%)	1320 (80.3%)	0.98 (0.63,1.52)		77 (79.4%)	1351 (80.4%)	0.94 (0.57,1.56)	
Difficulty breathing	30 (22.2%)	310 (18.9%)	1.23 (0.80, 1.88)		26 (26.8%)	314 (18.7%)	1.59 (1.00, 2.54)	3.35 (1.38,8.14)
Cough	91 (67.4%)	1000 (60.9%)	1.33 (0.92, 1.93)	1.48 (0.94,2.35)	68 (70.1%)	1023 (60.9%)	1.51 (0.97, 2.35)	1.93 (1.13,3.30)
Belly pain	89 (65.9%)	1035 (63.0%)	1.13 (0.78, 1.64)		58 (59.8%)	1066 (63.4%)	0.88 (0.58, 1.36)	
Vomit >3 times	75 (55.6%)	783 (47.7%)	1.37 (0.96, 1.95)	1.13 (0.78,1.65)	40 (41.2%)	818 (48.7%)	0.74 (0.49, 1.12)	
Irritable/ restless	99 (73.3%)	1159 (70.5%)	1.15 (0.77, 1.70)		67 (69.1%)	1191 (70.9%)	0.92 (0.59, 1.43)	
Decreased activity/ lethargy	73 (54.1%)	821 (50.0%)	1.18 (0.83, 1.67)		49 (50.5%)	48 (49.5%)	1.01 (0.67, 1.52)	
Lost consciousness	15 (11.1%)	133 (8.1%)	1.42 (0.81,2.50)		4 (4.1%)	144 (8.6%)	0.46 (0.17,1.27)	
Rectal straining	14 (10.4%)	145 (8.8%)	1.24 (0.70, 2.20)		4 (4.1%)	155 (9.2%)	0.43 (0.16, 1.18)	0.38 (0.14,1.08)
Rectal prolapse	3 (2.2%)	23 (1.4%)	1.61 (0.48, 5.44)		0 (0.0%)	26 (1.6%)	-	
Convulsions	6 (4.4%)	29 (1.8%)	2.59 (1.06, 6.35)	2.79 (1.11,7.05)	1 (1.0%)	34 (2.0%)	0.50 (0.07, 3.73)	
Median days of diarrhea	3	3	-		3	3	-	

Maximum number of stools passed in 24-hour period during illness up to enrollment*								
≤ 6	96 (71.1%)	1223 (74.4%)	ref.	ref.	67 (69.1%)	1252 (74.5%)	ref.	ref.
7-10	33 (24.4%)	365 (22.2%)	1.15 (0.76, 1.74)		26 (26.8%)	372 (22.1%)	1.31 (0.82, 2.08)	
>10	6 (4.4%)	55 (3.4%)	1.39 (0.58, 3.31)		4 (4.1%)	57 (3.4%)	1.31 (0.46, 3.72)	
Characteristics of stool sample provided at enrollment								
Contains mucus	96 (71.1%)	1155 (70.3%)	1.04 (0.71, 1.53)		73 (75.3%)	1178 (70.1%)	1.30 (0.81, 2.08)	
Watery‡	80 (59.3%)	989 (60.2%)	0.96 (0.67, 1.37)		57 (58.8%)	1012 (60.2%)	0.94 (0.62, 1.43)	
Bloody	4 (3.0%)	62 (3.8%)	0.87 (0.27, 2.83)		5 (5.2%)	61 (3.6%)	1.44 (0.57, 3.68)	
Contains pus	4(3.0%)	51 (3.1%)§	0.95 (0.34, 2.68)		1 (1.0%)	54 (3.2%)§	0.31 (0.04, 2.29)	

*Reported by caretaker; †Temperature >38°C measured in health facility; ‡Referent = stool was formed, soft, or thick liquid; § N=1642

Table 3. Relative risk by prevalence of breastfeeding in children <2 years old with MSD by EPEC status enrolled in GEMS, rural western Kenya, 2008-2011

	Typical EPEC			Atypical EPEC		
	Positive	Negative	Relative risk (95% CI)	Positive	Negative	Relative risk (95% CI)
Age 0-5 months	**30**	**216**		**17**	**229**	
Exclusively breastfed	3 (10.0%)	51 (23.6%)	0.42 (0.14,1.27)	4 (23.5%)	50 (21.8%)	0.91 (0.28,2.91)
Partially breastfed	25 (83.3%)	160 (74.1%)	1.12 (0.94,1.34)	13 (76.5%)	172 (75.1)	1.02 (0.77,1.34)
No breastfed	2 (6.7%)	5 (2.3%)	2.88 (0.58,14.19)	0 (0.0%)	7 (3.1%)	---
Age 6-11 months	**41**	**386**		**29**	**398**	
Exclusively breastfed	0 (0.0%)	6 (1.6%)	---	1 (3.5%)	5 (1.3%)	2.74 (0.33,22.72)
Partially breastfed	37 (90.2%)	359 (93.0%)	0.97 (0.87,1.08)	25 (86.2%)	371 (93.2%)	0.92 (0.80,1.07)
No breastfed	4 (9.8%)	21 (5.4%)	1.79 (0.65,4.97)	3 (10.3%)	22 (5.5%)	1.87 (0.60,5.89)
Age 12-23 months	**29**	**381**		**26**	**384**	
Exclusively breastfed	0 (0.0%)	1 (0.3%)	---	0 (0.0%)	1 (0.3%)	---
Partially breastfed	22 (75.9%)	279 (73.2%)	1.04 (0.84,1.28)	21 (80.8%)	280 (72.9%)	1.11 (0.91,1.35)
No breastfed	7 (24.1%)	101 (26.5%)	0.91 (0.47,1.77)	5 (19.2%)	103 (26.8%)	0.72 (0.32,1.60)

Includes GEMS-1 data collected from January 31, 2008 and January 28, 2011

Table 4. Enteric pathogens identified in the stool of children with MSD (n=1718) by EPEC status at enrollment, rural western Kenya, 2008-2012

	Typical EPEC		Atypical EPEC	
	Positive (N=131)	Negative (N=1587)	Positive (N=95)	Negative (N=1623)
Enteric pathogen				
Giardia	17 (13.0%)	300 (18.9%)	22 (23.2%)	295 (18.9%)
Rotavirus	16 (12.2%)	226 (14.2%)	15 (15.8%)	227 (14.0%)
Cryptosporidium	16 (12.2%)	171 (10.8%)	11 (11.6%)	176 (10.8%)
C. jejuni	12 (9.2%)	149 (9.4%)	14 (14.7%)	147 (9.1%)
Norovirus GII	8 (6.1%)	78 (4.9%)	2 (2.1%)	84 (5.2%)
Enteroaggregative *E. coli*	7 (5.3%)	252 (15.9%)	6 (6.3%)	253 (15.6%)
Adenovirus (not type 40/41)	6 (4.6%)	39 (2.5%)	2 (2.1%)	43 (2.7%)
Enterotoxigenic *E. coli* (LT only)	6 (4.6%)	93 (5.9%)	1 (1.1%)	98 (6.0%)
Shigella spp.	5 (3.8%)	121 (7.6%)	8 (8.4%)	118 (7.3%)
C. coli	5 (3.8%)	73 (4.6%)	2 (2.1%)	76 (4.7%)
Norovirus GI	4 (3.1%)	48 (3.0%)	1 (1.1%)	51 (3.1%)
Salmonella Non-Typhi	2 (1.5%)	91 (5.7%)	6 (6.3%)	87 (5.4%)
Astrovirus	2 (1.5%)	28 (1.8%)	5 (5.3%)	25 (1.5%)
Adenovirus (type 40/41)	5 (3.8%)	34 (2.1%)	1 (1.1%)	38 (2.3%)
Enterotoxigenic *E. coli* (ST or ST/LT)	1 (0.8%)	168 (10.6%)	1 (1.1%)	168 (10.4%)
Sapovirus	1 (0.8%)	53 (3.3%)	0 (0.0%)	54 (3.3%)
E. histolytica	1 (0.8%)	13 (0.8%)	1 (1.1%)	13 (0.8%)
V. cholera O1	0 (0.0%)*	7 (0.4%)	1 (1.1%)	6 (0.4%)
Aeromonas	0 (0.0%)*	1 (0.1%)	1 (1.1%)	0 (0.0%)*
Salmonella Typhi	0 (0.0%)*	0 (0.0%)*	0 (0.0%)*	0 (0.0%)*
Enterohemorrhagic *E. coli*	0 (0.0%)*	0 (0.0%)*	0 (0.0%)*	0 (0.0%)*

*Pathogen tested, but not identified

Table 5. Presentation of clinical symptoms in children with MSD (n=1717) at 60-90 day follow-up by EPEC status, rural western Kenya, 2008-2012

	Typical EPEC				Atypical EPEC			
	Positive (N = 131)	Negative (N = 1586)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Positive (N = 95)	Negative (N = 1622)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Mortality status								
Deceased	12 (9.2%)	50 (3.2%)	3.10 (1.61, 5.98)	2.87 (1.47,5.57)	4 (4.2%)	58 (3.6%)	1.19 (0.42, 3.34)	
Died at enrollment facility	2 (1.5%)	3 (0.2%)	8.22 (1.36, 49.63)		0 (0.0%)	5 (0.3%)	-	
Health status between enrollment and follow-up*								
Diarrhea	89 (67.9%)	1071 (67.5%)	1.02 (0.70, 1.49)		66 (69.5%)	1094 (67.5%)	1.10 (0.70, 1.72)	
Vomit	8 (13.8%)†	117 (15.9%)‡	0.85 (0.39, 1.83)		8 (15.7%)§	117 (15.8%)‖	0.99 (0.46, 2.17)	
Dysentery	1 (0.8%)	44 (2.8%)	0.27 (0.04, 1.97)		3 (3.2%)	42 (2.6%)	1.23 (0.37, 4.03)	
Cough	18 (31.0%)†	224 (30.5%)‡	1.03 (0.58, 1.83)		16 (31.4%)§	226 (30.5%)‖	1.04 (0.57, 1.92)	
Difficulty breathing	0 (0.0%)†	3 (0.4%)‡	-		1 (2.0%)§	2 (0.3%)‖	7.40 (0.66, 83.01)	

*Reported by caretaker; †N=58; ‡N=735; §N=58; ‖N=735; ¶Height-for-age <2 z-scores; **Weight-for-age <2 z-scores; ††N=119; ‡‡N=1529; §§N=1642

Table 6. Anthropometric indicators of malnutrition in children with MSD (n=1647) by EPEC status at enrollment and 60-90 day follow-up, rural western Kenya, 2008-2012

	Typical EPEC			Atypical EPEC		
	Positive (N = 119)	Negative (N = 1528)	Unadjusted OR (95% CI)	Positive (N = 91)	Negative (N = 1556)	Unadjusted OR (95% CI)
Enrollment						
HAZ <-2 z-scores	37 (31.1%)	404 (26.4%)	1.26 (0.84, 1.88)	27 (29.7%)	414 (26.6%)	0.78 (0.45, 1.36)
WAZ <-2 z-scores	30 (25.2%)*	319 (20.9%)	1.28 (0.83, 1.97)	16 (17.6%)	333 (21.4%)	1.16 (0.73, 1.85)
WHZ <-2 z-scores	16 (13.5%)†	155 (10.1%)	1.37 (0.79, 2.39)	11 (12.1%)	160 (10.3%)	1.20 (0.63, 2.30)
HAZ <-3 z-scores	11 (9.2%)‡	137 (9.0%)	1.03 (0.54, 1.97)	12 (13.2%)	136 (8.7%)	1.13 (0.51, 2.53)
WAZ <-3 z-scores	11 (9.2%)	102 (6.7%)	1.42 (0.74, 2.73)	7 (7.7%)	106 (6.8%)	1.59 (0.84, 2.98)
WHZ <-3 z-scores	7 (5.9%)	49 (3.2%)	1.88 (0.84, 4.26)	5 (5.5%)	51 (3.3%)	1.72 (0.67, 4.41)
Follow-up (60-90 days)						
HAZ <-2 z-scores	41 (34.5%)	527 (34.5%)	1.00 (0.67, 1.48)	33 (36.3%)	535 (34.4%)	1.09 (0.70, 1.69)
WAZ <-2 z-scores	20 (16.8%)*	262 (17.2%)	0.98 (0.59, 1.61)	13 (14.3%)	269 (17.3%)	0.80 (0.44, 1.46)
WHZ <-2 z-scores	9 (7.6%)†	109 (7.1%)	1.07 (0.53, 2.16)	8 (8.8%)	110 (7.1%)	1.27 (0.60, 2.69)
HAZ <-3 z-scores	18 (15.1%)‡	189 (12.4%)	1.26 (0.75, 2.13)	16 (17.6%)	191 (12.3%)	1.52 (0.87, 2.67)
WAZ <-3 z-scores	6 (5.0%)	76 (5.0%)	1.01 (0.43, 2.38)	5 (5.5%)	77 (5.0%)	1.12 (0.44, 2.83)
WHZ <-3 z-scores	4 (3.4%)	27 (1.8%)	1.93 (0.67, 5.62)	4 (4.4%)	27 (1.7%)	2.60 (0.89, 7.61)

Includes only cases with anthropometric measurements available at baseline and at 60-day follow-up.
*p-value=0.002; †p-value=0.02; ‡p-value=0.04

Table 7: Linear growth faltering among cases with and without EPEC (n=429) by age strata, rural western Kenya, 2008-2012

	Typical EPEC		Atypical EPEC	
	ΔHAZ*	p-value	ΔHAZ*	p-value

0-11 months	-0.22	0.002†	-0.05	0.57
12-23 months	0.05	0.67	-0.13	0.22
24-59 months	0.06	0.69	0.06	0.69

*ΔHAZ=Difference in mean height-for-age z-score (change in HAZ score from enrollment to follow-up) between cases with and without EPEC. †Significant difference, between cases with and without EPEC-positive MSD, p-value <0.05. Analysis controlled for duration of follow-up, base height-for-age z-score, number of days between enrollment and follow-up, and age at enrollment (in months)

Table 8. Environmental risk factors associated with EPEC MSD (n=1778) in children <5 years old reported at enrollment in GEMS, rural western Kenya, 2008-2012

	Typical EPEC				Atypical EPEC			
	Positive (N = 135)	**Negative (N = 1643)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**	**Positive (N = 97)**	**Negative (N = 1681)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
Primary source of drinking water*								
Rainwater	43 (31.9%)	588 (35.8%)	ref.		30 (30.9%)	601 (35.8%)	ref.	
Surface water	54 (40.0%)	609 (37.1%)	1.21 (0.80, 1.84)		43 (44.3%)	620 (36.9%)	1.39 (0.86, 2.24)	
Other improved water sources	33 (24.4%)	371 (22.6%)	1.22 (0.76, 1.95)		20 (20.6%)	384 (22.8%)	1.04 (0.58, 1.86)	
Other unimproved water sources	5 (3.7%)	75 (4.6%)	0.91 (0.35, 2.37)		4 (4.1%)	76 (4.5%)	1.05 (0.36, 3.08)	
Always available from main source	6 (4.4%)	125 (7.6%)	0.56 (0.24, 1.31)		6 (6.2%)	125 (7.4%)	0.82 (0.35, 1.91)	
Child drinking water habits within 2 weeks prior to enrollment								
Child drank untreated water	16 (18.4%)§	327 (31.6%)‖	0.49 (0.28, 0.85)	0.55 (0.31, 1.0)	15 (23.8%)‖‖	328 (30.9%)¶¶	0.70 (0.39, 1.26)	
Child given stored drinking water	114 (84.4%)	1500 (91.3%)	0.52 (0.32, 0.85)	0.61 (0.30,1.21)	88 (90.7%)	1526 (90.8%)	0.99 (0.49, 2.01)	
Household drinking water treatment†								
Treats drinking water	87 (64.4%)	1037 (63.1%)	1.06 (0.73, 1.53)		63 (65.0%)	1061 (63.1%)	1.08 (0.71, 1.66)	
Effective treatment method	81 (60.5%)¶	987 (63.8%)**	0.87 (0.61, 1.25)		58 (63.0%)***	1010 (63.5%)†††	0.98 (0.63, 1.51)	
Chlorine	69	838	-		50	857	-	
Boils	12	145	-		8	149	-	
Observed Sanitation Facilities								
Has facility for feces disposal	96 (73.3%)‡†	1157 (72.9%)‡‡	1.02 (0.68, 1.52)		75 (79.0%)‡‡‡	1178 (72.6%)§§§	1.42 (0.85, 2.35)	
Traditional pit toilet	90	1049	-		70	1069	-	
VIP latrine	6	106	-		5	107	-	
Visible feces in compound	45 (33.3%)	609 (37.1%)	0.85 (0.59, 1.23)		40 (41.2%)	614 (36.5%)	1.22 (0.80, 1.85)	
Hand Hygiene‡								
Washes hands before:								
Eating	102 (75.6%)	1378 (83.9%)	0.59 (0.39, 0.90)	0.70 (0.41,1.19)	81 (83.5%)	1399 (83.2%)	1.02 (0.59, 1.77)	

Nursing	47 (34.8%)	491 (29.9%)	1.25 (0.87, 1.81)		34 (35.1%)	504 (30.0%)	1.26 (0.82, 1.94)
Cooking	45 (33.3%)	550 (33.5%)	0.99 (0.68, 1.44)		33 (34.0%)	562 (33.4%)	1.03 (0.67, 1.58)
Washes hands after:							
Defecating	115 (85.2%)	1260 (76.7%)	1.75 (1.07, 2.85)	1.40 (0.75,2.59)	77 (79.4%)	1298 (77.2%)	1.14 (0.69, 1.88)
Cleaning child	39 (28.9%)	422 (25.7%)	1.18 (0.80, 1.73)		27 (27.8%)	434 (25.8%)	1.11 (0.70, 1.75)
Handling animals	18 (13.3%)	184 (11.2%)	1.22 (0.73, 2.05)		9 (9.3%)	193 (11.5%)	0.79 (0.39, 1.59)
Wash with soap	58 (44.3%)[5]	829 (52.3%)[§§]	0.73 (0.51, 1.04)		54 (56.8%)[§§§]	833 (51.4%)[¶¶¶]	1.25 (0.82, 1.89)
Animal Ownership							
Present in compound	132 (97.8%)	1635 (99.5%)	0.22 (0.56, 0.82)	0.21 (0.04,1.13)	95 (97.9%)	1672 (99.5%)	0.26 (0.05, 1.20)
Ruminant animal	94 (69.6%)	1281 (80.0%)	0.65 (0.44, 0.95)		71 (73.2%)	1304 (77.6%)	0.79 (0.50, 1.26)
Wealth Index Quintile							
First quintile (poorest)	21 (15.6%)	291 (17.7%)	ref.		11 (11.3%)	301 (17.9%)	ref.
Second quintile	31 (23.0%)	335 (20.4%)	1.28 (0.72, 2.28)		27 (27.8%)	339 (20.2%)	2.18 (1.06, 4.47)
Third quintile	31 (23.0%)	434 (26.4%)	0.99 (0.56, 1.76)		19 (19.6%)	446 (26.5%)	1.17 (0.55, 2.48)
Fourth quintile	26 (19.3%)	265 (16.1%)	1.36 (0.75, 2.47)		20 (20.6%)	271 (16.1%)	2.02 (0.95, 4.29)
Fifth quintile (wealthiest)	26 (19.3%)	318 (19.4%)	1.13 (0.62, 2.06)		20 (20.6%)	324 (19.3%)	1.69 (0.80, 3.58)

*In the two weeks prior to enrollment; reported by caretaker. †Treatment methods caretakers report using most often when treating water. ‡Reported by caretaker; without probing from the questionnaire administrators. §N=87; ||N=1036; ¶N=134; **N=1548; ††N=131; ‡‡N= 1587; §§N=1586; ||||N=63; ¶¶N=1060; ***N=92; †††N=1590; ‡‡‡N=95; §§§N= 1623; ¶¶¶N=1622

Appendix I

		DIARRHEA 	NORMAL 
(1)	☼ (today)	X	
(2)	☼☼	X	
(3)	☼☼☼	X	
(4)	☼☼☼☼	X	
(5)	☼☼☼☼☼	X	
(6)	☼☼☼☼☼☼		
(7)	☼☼☼☼☼☼☼		
(8)	☼☼☼☼☼☼☼☼		
(9)	☼☼☼☼☼☼☼☼☼		
(10)	☼☼☼☼☼☼☼☼☼☼		
(11)	☼☼☼☼☼☼☼☼☼☼☼		
(12)	☼☼☼☼☼☼☼☼☼☼☼☼		
(13)	☼☼☼☼☼☼☼☼☼☼☼☼☼		
(14)	☼☼☼☼☼☼☼☼☼☼☼☼☼☼		