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Diarrhea, enteric pathogen detection, and nutritional indicators among controls in the Global Enteric Multicenter Study, Kenya site: An opportunity to understand reference populations in case-control studies of diarrhea.

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Summary:

Given the challenges in accurately identifying unexposed controls in case-control studies of diarrhea, we examined diarrhea incidence, subclinical enteric infections, and growth stunting within a reference population in the Global Enteric Multicenter Study, Kenya site. Within "control" children (0-59 month-olds without diarrhea in the 7-days before enrollment, n=2,384), we examined surveys at enrollment and 60-day follow-up, stool at enrollment, and a 14-day post-enrollment memory aid for diarrhea incidence. At enrollment, 19% of controls had ≥1 enteric pathogen associated with moderate-to-severe diarrhea ("MSD pathogens") in stool; following enrollment, many reported diarrhea (27% in 7 days, 39% in 14 days). Controls with and without reported diarrhea had similar carriage of MSD pathogens at enrollment; however, controls

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reporting diarrhea were more likely to report visiting a health facility for diarrhea (27% vs. 7%)or fever (23% vs. 16%) at follow-up than controls without diarrhea. Odds of stunting differed by both MSD and “any” (including non-MSD pathogens) enteric pathogen carriage, but not diarrhea, suggesting control classification may warrant modification when assessing long-term outcomes. High diarrhea incidence following enrollment and prevalent carriage of enteric pathogens have implications for sequelae associated with subclinical enteric infections and for design and interpretation of case-control studies examining diarrhea.

Introduction

Globally, over 1.7 billion children are affected each year by diarrhea [1], an important—yet complex—health condition. Across numerous published studies [2], measurement of diarrhea varies from self-report to confirmed clinical and laboratory diagnoses [3]. Even the most detailed studies fail to identify the etiologic agent in all cases, but clinical and laboratory data now exist to estimate pathogen-specific disease burdens. Diarrhea can be caused by various infectious agents—bacteria, viruses, protozoa, and soil-transmitted helminths—that differ in their relative contribution to diarrheal morbidity and mortality [3–5]. These organisms also vary in their incubation period, the probability with which symptoms occur following exposure, and the duration during which the organism is excreted in feces after symptoms resolve [6]. During epidemiologic studies of diarrheal diseases, these variations make it difficult to accurately identify unexposed controls and to identify the precise cause of acute symptoms when multiple pathogens are identified in stool testing.

Case-control studies with laboratory testing of stool specimens are common designs for ascertaining etiologic agents [7–10] and assessing pathogen-specific disease burden and risk factors [11, 12]. Case and control definitions that employ specific clinical criteria allow for more accurate classification of disease severity and health status, and a more precise outcome measure [13]. Often control eligibility is restricted by clinical criteria, such as the absence of diarrheal symptoms in the control for a defined period. As mild diarrheal illness in young children is common in developing countries, imperfect recall may lead to misclassification of children convalescing from an episode of diarrheal disease or incubating diarrheal disease as controls. [14–16]. Moreover, as cases are often enrolled in health facilities while controls are enrolled in the community, specimen collection from controls and transport to a laboratory for confirmation of control (non-diseased) status is challenging and may yield a higher proportion of false negative tests, given that asymptomatic individuals often produce fewer pathogens per gram of stool [6]. Further, logistical constraints in case-control studies often restrict contact with controls to a single visit at enrollment, where both inclusion criteria and risk factors are ascertained [9–11]. Follow-up to confirm disease-free status is rarely attempted.

Because of the challenges in accurately identifying unexposed controls in case-control studies of diarrhea, and the growing recognition of subclinical enteric infections as a determinant of longer-term health outcomes, we sought to examine the incidence of diarrhea, subclinical enteric infections, and growth stunting within a reference population. The Global Enteric Multicenter Study (GEMS) — a multisite case-control study of

moderate-to-severe diarrhea (MSD) in children < 5 years old in Africa and south Asia [17]—provides a unique opportunity to examine diarrhea incidence, enteric pathogen prevalence, and longer term outcomes including growth stunting, in a control population. The goal of this study was to characterize the health of controls in the GEMS study following enrollment, including diarrheal symptoms, enteric pathogen detection in stool, and stunting. Studying controls can reveal background rates of diarrhea and enteric pathogen carriage, and inform future criteria for control selection in diarrheal disease studies.

Methods

GEMS was a matched case-control study of MSD in children <5 years old, conducted in seven sites in sub-Saharan Africa and South Asia to improve understanding of the etiology and burden of diarrheal diseases in low-income settings [17]. This analysis focuses on GEMS data collected at the Kenya study site [5, 18–21].

Study site

The GEMS Kenya site, located in rural, western Kenya, has been described previously [13, 18–21]. The population enrolled at the Kenya site participated in a health and demographic surveillance system (HDSS) that visited each household thrice annually to obtain information about births, deaths, migration, and other factors. Children were enrolled between January 31, 2008-January 29, 2011 and between October 31, 2011-September 30, 2012.

Inclusion criteria for controls

Control children matched by age, sex, and neighborhood were randomly selected from the HDSS population and visited at home within 14 days of case identification. Controls were enrolled if their caretaker reported the child was free of diarrhea for 7 days before the visit, and consented to participation. Detail on sampling frame and case-control selection are described elsewhere [13].

Enrollment and follow-up

At enrollment, a questionnaire was administered to determine each child's eligibility as a control. A stool specimen was obtained from each eligible consented child, delivered to the lab, and processed within 18 hours of enrollment. A questionnaire concerning household demographics; socio-economic status; water, sanitation, and hygiene (WASH) conditions; and feeding and other medical conditions of the child was administered to caretakers, and the child's length/height was measured. Finally, the caretaker was given a 14-day memory aid form to record daily diarrheal incidence and was instructed that enumerators would return in approximately 60 days (acceptable window: 49–91 days) to conduct a follow-up visit. At the 60-day visit, the memory aid form was collected, data on illness and healthcare seeking for the child subsequent to enrollment were collected, and anthropometric measurements were repeated.

Stool collection at enrollment

All stool specimens from controls underwent the same methods of collection, transport, delivery to the lab, and testing for the spectrum of bacterial, viral, and parasitic enteric pathogens via conventional microbiological methods as specimens from cases [22].

Administration of the 14-day memory aid form

A memory aid for daily incidence of diarrhea was created for the caretaker of cases and control children to complete during the 14 days following enrollment [13, 18]. Caretakers were trainedin the definition of diarrhea used—passage of ≥3 loose or watery stool in the previous 24 hours—and instructed to fill the form daily. At the 60-day visit, the memory aid was reviewed with the caretaker to resolve any unclear or missing data. We defined “any diarrhea” as ≥1 day of diarrhea denoted on the memory aid within the 14-day period after enrollment. Incidence was also broken down by date of onset post-enrollment.

Anthropometry

Anthropometric measurements (length/height) were collected for controls at home at enrollment and follow-up as described previously [13] using a “Shorr board.” Height-for-age Z-scores (HAZ) were calculated using a WHO SAS macro and the WHO Child Growth Standards for the reference population [23, 24]. Staff performing measurements underwent a training and quality assessment regimen for the duration of the study, as previously described [13]. To mitigate the impact of measurement error, outliers defined by both WHO [24] and using median absolute deviation (MAD) methods [25] were censored. HAZ scores were calculated to assess stunting for each child at enrollment and 60-day follow-up based on standard WHO stunting criteria (<−2 z-scores).

Statistical Analysis

Data were stored and managed in SAS software version 9.4 (SAS, Cary, NC, USA) and analyses conducted in R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria, [26]). We performed logistic regression to compare diarrhea and enteric pathogen detection in controls. We categorized controls by a) development of any/no reported diarrhea (from memory aid data); b) detection in stool collected at enrollment of any/no MSD enteric pathogen (defined as pathogens significantly associated with MSD at the Kenya site − rotavirus, *Cryptosporidium*, *Shigella* spp., typical enteropathogenic *E. coli* (tEPEC), heat-stable-toxin-producing enterotoxigenic *E. coli* (ST-ETEC,) and non-typhoidal *Salmonella* spp. [5]); c) detection in stool at enrollment of any/no potential enteric pathogens (defined as any pathogens tested from the entire list of GEMS pathogens assessed in the stool specimen at enrollment, listed in Table S2 [22]); and d) four distinct groups based on diarrhea and MSD pathogen detection: diarrhea +/pathogen + (G1), diarrhea −/pathogen + (G2), diarrhea +/pathogen - (G3), and diarrhea −/pathogen - (G4) groups (Table S1b). G1 and G3 were combined in subsequent analyses to measure children with diarrhea against children without reported diarrhea but with pathogens detected (G2) and children without reported diarrhea or pathogens detected (G4).

Logistic regression models were run with dummy variables for levels of the previously-described subgroups as predictors, and clinical, health, WASH conditions, and stunting

as outcomes to investigate differences between groups. Age group (0–11, 12–23, 24–59 months) and sex were considered potential effect modifiers in all models and reported if significant at alpha = 0.05. Age groups and sex were included in models when effect modification was not present (all p-values for interaction with age or sex > 0.05) and adjusted estimates are reported.

At the GEMS Kenya site, 125 (4.9%) controls were enrolled more than once. To examine their influence on the results, sensitivity analyses excluding repeat enrollments were conducted.

Ethics

The study was reviewed and approved by the KEMRI Scientific and Ethical Review Committees (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 CDC, Atlanta, GA deferred its review to the University of Maryland IRB (CDC Protocol #5038).

Results

Demographics, diarrhea, and detection of enteric pathogens in controls

Of the 2,534 controls in the GEMS Kenya site with follow-up during the acceptable window, 2,384 (94%) had a completed memory aid for diarrhea recall; we excluded from further analysis the 150 (6%) who did not (Table S1a). Among controls with a completed memory aid, mean age was 18 months (range 0–59 months old (mo)), 36% were infants (0–11 mo), and 57% were male (Table 1). Controls that did not have a completed memory aid form did not differ significantly in age or sex from those included (data not shown). Among the 919 (39%) controls that developed “any diarrhea”, onset clustered soon after enrollment and peaked on Day 3 (132 (14%), Figure 1), with 643 (27% of all controls, 70% of controls with diarrhea) reporting onset by Day 7 (Table 1). Children 24–59 mo had lower reported diarrhea incidence (31%) than those 0–11 or 12–23 mo (42%).

At least one MSD enteric pathogen was detected in 460 stool specimens collected from controls at enrollment (19%); detection rates decreased by age group (Table 1). The most prevalent MSD pathogens were tEPEC (4.8%), ST-ETEC (4.2%), and *Cryptosporidium* (4.1%, Table S2). Co-detection of MSD pathogens was uncommon (2%). Approximately 68% of controls’ stool specimens at enrollment had at least one potential enteric pathogen detected, most commonly *Giardia* spp. (24%) and enteroaggregative *E. coli* (16%, Table S2).

Adjusting for age and sex, detection of tEPEC was higher in controls that developed diarrhea than in those that did not (OR: 1.5, 95% CI: 1.0–2.1, p = 0.05, Table S2).

Health outcomes and WASH exposures by diarrhea and pathogen detection

Controls that did and did not develop any diarrhea did not vary significantly in detection of any MSD enteric pathogens in stool collected at enrollment (Table 2). Controls that developed diarrhea had significantly higher odds of reporting fever in the week preceding enrollment (OR: 1.6, 95% CI: 1.4–1.9) and of having used an unimproved water source

(OR: 1.3, 95% CI: 1.1–1.5) than controls that did not develop diarrhea. At 60-day follow-up, controls that developed diarrhea had significantly higher odds of having visited a health facility for diarrhea (OR: 4.9, 95% CI: 3.8–6.4), having had fever (OR: 1.8, 95% CI: 1.5–2.2), and having visited a health facility for fever (OR: 1.5, 95% CI: 1.2–1.9). Overall, 71% (253) of controls who reported having sought care for diarrhea at the 60-day follow-up visit had reported diarrhea on the memory aid. Only 101 (7%) of the 1,465 controls who did not report diarrhea on the memory aid reported having sought care for diarrhea at the 60-day follow-up visit. Male controls that developed diarrhea were significantly more likely to report having had dysentery in the last 60 days (OR: 16.9, 95% CI: 2.2–132), but female controls were not.

Although few deaths (13) were observed in control children, those with MSD pathogens detected in stool at enrollment were more likely to have died by 60-day follow-up than those without MSD pathogens (6/460 (1.3%) vs. 7/1924 (0.4%), OR: 3.2, 95% CI: 1.0–9.7, Table 3). Five of six deaths in control children with MSD pathogens were amongst those who reported developing diarrhea (data not shown). No other significant differences were observed between controls with/without MSD pathogens detected. Controls with and without any potential enteric pathogens detected in stool at enrollment did not differ significantly in health or WASH conditions at enrollment, or health at 60-day follow-up (Table S4).

Differences in health conditions in controls by diarrhea-enteric pathogen group

When controls were divided by both reported diarrhea and MSD pathogen detection, 198 (8.3%) reported diarrhea and had an MSD pathogen detected (G1), 262 (11%) did not report diarrhea but had an MSD pathogen detected (G2), 721 (30%) reported diarrhea but did not have an MSD pathogen detected (G3), and 1203 (51%) did not report diarrhea or have an MSD pathogen detected (G4, Table S1b). G1 and G3 controls tended to have similar health conditions when measured descriptively (Table S5). Differences in clinical conditions were assessed for combined diarrheal controls (G1+G3 controls), non-diarrheal controls with MSD pathogens detected (G2), and non-diarrheal controls without MSD pathogens detected (G4, Table 4). G1+G3 controls had significantly higher odds of having a fever (OR: 1.7, 95% CI: 1.4–2.0) or vomiting (OR: 1.7, 95% CI: 1.0–3.0) in the 7 days preceding enrollment compared to G4 controls.

At 60-day follow-up, G1+G3 controls had higher odds of having visited a health facility for diarrhea (OR: 4.8, 95% CI: 3.7–6.3) or having had dysentery (OR: 3.8, 95% CI: 1.5–10.6) during the follow-up period than G4 controls. G1+G3 controls also had higher odds of fever (OR: 1.8, 95% CI: 1.5–2.1) or of having visited a health facility for fever (OR: 1.5, 95% CI: 1.2–1.8) during the follow-up period than G4 controls. G2 and G4 controls did not differ significantly in health outcomes at follow-up.

Exclusion of the 125 (4.9%) control children with repeat enrollments did not appreciably change the results of our analyses (data not shown).

Stunting in controls by presence of diarrhea and detection of enteric pathogens, adjusted for age and sex

Controls that did and did not develop any diarrhea did not vary significantly in odds of stunting at enrollment or follow-up (Table 5a). Controls with MSD enteric pathogens detected in stool (both with and without diarrhea) did not differ from controls without an MSD enteric pathogen in odds of being stunted at enrollment, but had significantly higher odds of being stunted at 60-day follow-up (OR: 1.6, 95% CI: 1.1–2.2, Table 5b). Conversely, controls with any potential pathogen detected in stool had significantly higher odds of being stunted at enrollment (OR: 1.3, 95% CI: 1.1–1.6), but not at 60-day follow-up, compared with controls without a potential pathogen detected. Controls did not differ in odds of stunting by G1-4 designations of diarrhea/MSD pathogen status (Table 5c).

Discussion

Among control children in the GEMS Kenya site, we found significant carriage of enteric pathogens associated with MSD (19%) and of any potential enteric pathogen (68%) at enrollment, and high incidence of diarrhea soon after enrollment (27% within 7 days, 39% within 14 days). At follow-up, 28% of controls that reported developing diarrhea on the memory aid had sought healthcare for diarrhea, compared with only 7% of controls who had not reported developing diarrhea. No data were collected that would allow episodes of diarrhea among controls to be classified as MSD, but some that were severe enough to warrant a visit to a health facility may have met the GEMS case criteria. Controls with enteric pathogens detected in stool—with or without diarrhea—had higher odds of stunting than those that did not have an enteric pathogen detected, suggesting analysis of such longer-term outcomes may require case definitions inclusive of mild diarrhea or subclinical infections.

This study is the first, to our knowledge, to separately examine the gastrointestinal health — including symptomatic and subclinical infection — of study controls at enrollment, during the 14 days following enrollment, and at 60-day follow-up. Eligibility criteria for GEMS controls required the child to have been free from diarrhea in the preceding 7 days, as is common practice in case-control studies of diarrhea [7–9, 11, 12]. Few case-control studies have collected such detailed data on a reference population, including a) stool specimens at enrollment tested for the same comprehensive panel of enteric pathogens as case stool specimens; b) a daily record of diarrhea during the 14 days post-enrollment; and c) 60-day follow-up visits to repeat anthropometric measurements and enquire about illness subsequent to enrollment. These additional data allow a more detailed characterization of the referent population than is usually afforded.

The prevalence of enteric pathogens detected at enrollment and incidence of diarrhea following enrollment suggest that a substantial proportion of this control population had either residual or incubating subclinical infection during the study period [27]. Alternatively, certain enteric pathogens detected in control stool specimens (e.g. ETEC or EPEC) may have “colonized” the large intestine but lacked the signals within the intestinal environment required to activate virulence gene expression or previously acquired infection-derived immunity [6]. The high incidence of diarrhea shortly after enrollment is an important

indicator of active infection that may have been incubating at enrollment: in particular, in the 27% of controls who had diarrhea within 7 days after enrollment (70% of controls with diarrhea) and especially in the 10% of all controls who visited a health facility for diarrhea between enrollment and follow-up. Controls who developed developing diarrhea and experienced subsequent symptoms (fever, dysentery) that led them to seek care at a health center could have had other host or environmental factors that predisposed them to more symptomatic or recurrent diarrhea, besides the diarrheal episode reported on the memory. However, without repeat fecal microbiology at the time of diarrhea onset and a comparator population that allows for adjustment of potential confounders, a causal relationship between reported diarrhea on the memory aid and subsequent symptoms (fever) and care-seeking at the 60-day follow-up visit cannot be determined with certainty.

Data on the frequency of detection of each enteric pathogen, and on episodes of diarrhea in controls, are necessary to more precisely identify risk factors for diarrheal pathogen-specific illness, and to estimate the fraction of MSD attributable to each pathogen. GEMS investigators applied enteric pathogen prevalence data from controls in pathogen-specific attribution estimates [5, 28], but data on the frequency of diarrhea among controls has not yet been used to improve their accuracy. Controls found to have evidence of recent infection are often excluded from risk factor analyses [29, 30] to avoid misclassification and bias towards the null. Although total MSD pathogen carriage among controls was 19%, carriage of any single pathogen associated with MSD in the GEMS Kenya site did not exceed 5%, suggesting little risk of bias in the original calculations of attributable fraction.

Recent evidence suggests that subclinical enteric infections may have detrimental effects on long-term development in children, such as stunting, independent of diarrhea [3, 31]. Data from this study are consistent with this previous evidence: controls with carriage of any potential enteric pathogen had a higher odds of stunting at enrollment compared to those without carriage of any potential enteric pathogen; those with carriage of MSD pathogens had a higher odds of stunting at follow-up compared to those without carriage of MSD pathogens, while reported diarrhea was not significantly associated with stunting among controls. While interpretation of differences from our study is limited given the case-control study design and short follow-up period (60 days), previous evidence suggests that repeat symptomatic and subclinical infections may lead to environmental enteric dysfunction (EED), a state of chronic inflammation of the gut [3, 32–35]. Evidence that EED may act independently of diarrhea prevalence has been observed in studies employing a longer follow-up period [36], including a multisite birth cohort of children 0–2 years of age [37]. These data, combined with results from this study, suggest assessment of enteric pathogen carriage should accompany measurement of diarrhea when evaluating long-term outcomes such as linear growth.

Timing of outcome onset may be important in reducing outcome misclassification, as up to 10% of all controls (including >25% of controls reporting diarrhea) in this analysis may have qualified as cases within the 60-day follow-up period. Because extending the period when potential controls must be absent diarrhea prior to enrollment may be both logistically challenging and present concerns of recall bias, an alternative strategy of disaggregating

controls into subgroups based on clinical variation may be more feasible, with subsequent analysis targeting symptom- and pathogen-free controls as necessary.

This study has limitations. First, because GEMS is a tightly matched case-control study, the results from controls are not generalizable to the entire study population, and implications should be limited to reference populations in case-control studies. Second, detection of enteric pathogens in stool at enrollment does not provide information about the timing or association with symptom onset, limiting conclusions about the etiologic cause of reported diarrhea. Though reported diarrhea has a well-documented potential for bias with varying recall periods [14–16], use of a memory-aid form filled daily [18] may have minimized these issues. However, reduced incidence of reported diarrhea in the second week of memory aid documentation (Fig. 1) may also suggest that caregivers' adherence to filling the form decreased over time. Of note, the use of laboratory tests with high sensitivity to potentially low pathogen loads in individuals without diarrhea (e.g. controls) in GEMS was a study strength [27].

It is important that future studies of enteric infection and diarrhea, especially case-control designs like GEMS, continue to employ sensitive enrollment and follow-up measures—including potential assessment of underlying or subsequent subclinical enteric infections through molecular diagnostics—to minimize misclassification and contextualize study results with regard to background levels of infection. Given recent progress in diagnostic techniques, including multiplex PCR [38, 39], improved characterization of study outcomes from stool specimens is becoming more feasible in LMICs. Additionally, the use of a memory-aid form or other, similar method may improve capture of symptom onset after enrollment [18].

This analysis of control children in the GEMS Kenya site, who reported no diarrhea in the week preceding enrollment, revealed that many had underlying residual, concurrent, or incubating enteric infection or colonization. Some of these may have been subclinical infections and a significant number went on to have diarrhea in the following 2 weeks. Odds of stunting varied significantly by detection of enteric pathogens in stool, regardless of diarrheal symptoms, which is in agreement with other, multisite birth cohort studies [37] underscoring the importance of measuring enteric pathogen carriage in stool in addition to diarrheal outcomes. This variation in both short- and long-term health outcomes in control children underscores the importance of extending the use of sensitive metrics for case status to controls to better understand their health status and more accurately characterize the study reference group.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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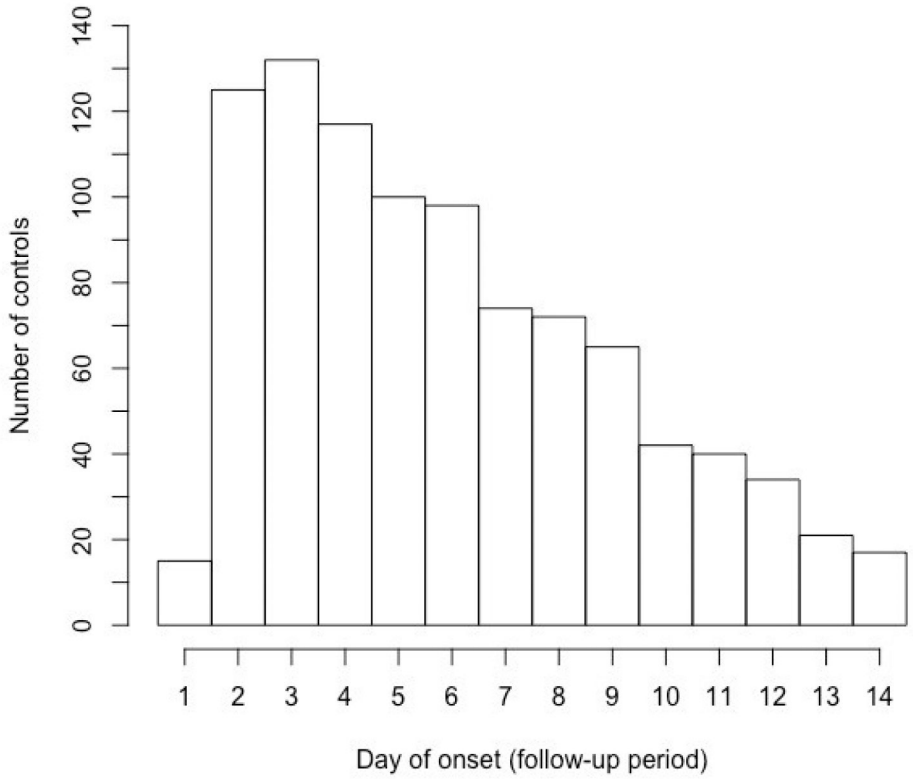

Figure 1:
Date of onset of diarrhea during 14-day Memory Aid period among controls with reported diarrhea

Table 1:

Demographics, diarrhea, and enteric pathogen detection in control children[1] at enrollment, Global Enteric Multicenter Study, Kenya site

Attribute	0–11 month-olds	12–23 month-olds	24–59 month-olds	Total (%)
Age group	856 (35.9%)	794 (33.3%)	734 (30.8%)	2,384 (100%)
Male	506 (59.1%)	456 (57.4%)	386 (52.6%)	1,348 (56.5%)
Any diarrhea within 7 days after enrollment	260 (30.3%)	211 (26.6%)	172 (23.4%)	643 (27.0%)
Any diarrhea within 14 days after enrollment	359 (41.9%)	330 (41.6%)	230 (31.3%)	919 (38.6%)
Days with diarrhea among all controls, median (range)	0 (0–11)	0 (0–13)	0 (0–11)	0 (0–13)
Among only controls with diarrhea, median (range)	3 (0–11)	3 (0–13)	3 (0–11)	3 (0–13)
MSD enteric pathogen[2] detected in stool at enrollment	192 (22.4%)	171 (21.5%)	97 (13.2%)	460 (19.3%)
# MSD enteric pathogens[2] detected in stool at enrollment, median (range)	0 (0–3)	0 (0–2)	0 (0–2)	0 (0–3)
Any potential enteric pathogen[3] detected in stool at enrollment	597 (69.7%)	563 (70.9%)	469 (63.9%)	1,629 (68.3%)
# potential enteric pathogens[3] detected in stool at enrollment, median (range)	1 (0–5)	1 (0–5)	1 (0–5)	1 (0–5)

[1] N = 2,534 children enrolled as controls; however, n = 2,384 control children that submitted a completed 14-day memory aid form;

[2] Any pathogens detected in a child's stool specimen at enrollment that were significantly associated with moderate-to-severe diarrhea (MSD) at the GEMS Kenya site (Rotavirus, *Cryptosporidium*, *Shigella* spp., typical enteropathogenic *E. coli* (tEPEC), heat-stable-toxin-producing enterotoxigenic *E. coli* (ST-ETEC,) and non-Typhoidal *Salmonella* spp.) [5].

[3] Any pathogens detected from the entire list of potential pathogens assessed in GEMS (Table S2) [22]

Table 2:

Analysis of controls with/without any diarrhea reported in 14-day memory aid form, Global Enteric Multicenter Study, Kenya site

Parameter	Controls with any diarrhea[1] N = 919	Controls without any diarrhea[1] N=1465	aOR[2]	p-value[2]
a) Health conditions at enrollment				
Detection of an MSD enteric pathogen[3] in stool	198 (21.5%)	262 (17.9%)	1.20 (0.97, 1.47)	0.090
Median # MSD enteric pathogens[3] detected (interquartile range)[4]	0 (0–0)	0 (0–0)	1.16 (0.97, 1.39)	0.098
Detection of any potential enteric pathogen[5] in stool	635 (69.1%)	993 (67.8%)	1.03 (0.86, 1.23)	0.784
Median # potential enteric pathogens[5] detected (interquartile range)[4]	1 (0–2)	1 (0–2)	1.02 (0.94, 1.10)	0.709
Blood in stool collected	0	4 (0.3%)	-	-
Blood in stool (in last 7 days)	2 (0.2%)	4 (0.3%)	0.79 (0.11, 4.09)	0.783
Fever (in last 7 days)	401 (43.6%)	480 (32.8%)	**1.62 (1.37, 1.93)**	< **0.001**
Vomiting (in last 7 days)	33 (3.6%)	36 (2.5%)	1.48 (0.91, 2.40)	0.115
b) Water, sanitation, and hvaiene conditions at enrollment				
Any sanitation facility present	697 (75.8%)	1106 (75.5%)	1.04 (0.86, 1.26)	0.708
Unimproved water source[6]	361 (39.3%)	492 (33.6%)	**1.25 (1.05, 1.49)**	**0.010**
Water treated	521 (56.7%)	820 (56.0%)	1.02 (0.86, 1.20)	0.828
Water treated effectively[7]	496 (54.0%)	771 (52.6%)	1.05 (0.89, 1.23)	0.603
Water treated with chlorine	433 (47.1%)	648 (44.2%)	1.13 (0.95, 1.33)	0.158
c) Health at 60-day follow-up				
Visited health facility for diarrhea in last 60 days	253 (27.5%)	101 (6.9%)	**4.92 (3.84, 6.36)**	**<0.001**
Dysentery in last 60d	16 (1.7%)	8 (0.6%)		
Females			1.34 (0.42, 4.25)	0.625
Males			**16.9 (2.18, 132)**	**0.007**
Visited health facility for dysentery in last 60 days	8 (0.9%)	4 (0.3%)	3.27 (1.02, 12.3)	0.055
Fever in last 60d	606 (66.2%)	745 (51.3%)	**1.83 (1.54, 2.17)**	< **0.001**
Visited health facility for fever in last 60 days	209 (22.7%)	234 (16.0%)	**1.52 (1.23, 1.87)**	< **0.001**
Death of child	8 (0.9%)	5 (0.3%)	2.30 (0.76, 7.66)	0.146

Bold indicates significant at 0.05.

[1] Based on responses in 14-day memory aid;

[2] Adjusted for age group and sex, stratified estimates by age group or sex presented where effect modification significant at 0.05 was observed;

[3] Any pathogens detected in a child's stool specimen at enrollment that were significantly associated with moderate-to-severe diarrhea (MSD) at the GEMS Kenya site [5].

[4] Modeled by multivariable Poisson regression. All other estimates by multivariable logistic regression;

[5] Any pathogens detected from the entire list of potential pathogens assessed in GEMS [22]

[6] Water source that does not meet the criteria for "improved," per the Joint Monitoring Program criteria [40] of a source that is safely protected from outside contamination (especially feces) via its construction or intervention;

[7] Effective water treatment classified as solar disinfection, chlorine disinfection, boiling, or filtration through ceramic or other filter. Ineffective water treatment classified as filtration through a cloth, alum, or other chemical added;

Table 3:

Analysis of controls with/without MSD enteric pathogen detected in stool at enrollment, Global Enteric Multicenter Study, Kenya site

Parameter	≥ 1 MSD enteric pathogens[1] detected N = 460	0 MSD enteric pathogens[1] detected N=1924	aOR[2]	p-value[2]
a) Health conditions at enrollment				
Blood in stool collected	0 (0%)	4 (0.2%)		
Blood in stool (in last 7 days)	1 (0.2%)	5 (0.3%)		
Fever (in last 7days)	179 (38.9%)	702 (36.5%)	1.12 (0.91, 1.38)	0.287
Vomiting (in last 7days)	16 (3.5%)	53 (2.8%)	1.23 (0.67, 2.14)	0.474
b) Water, sanitation, and hvaiene conditions at enrollment				
Any sanitation facility present	359 (78.0%)	1444 (75.1%)	1.20 (0.94, 1.54)	0.139
Unimproved water source[3]	167 (36.3%)	686 (35.7%)	1.00 (0.81, 1.24)	0.998
Water treated	276 (60.0%)	1065 (55.4%)	1.20 (0.97, 1.48)	0.091
Water treated effectively[4]	258 (56.1%)	1009 (52.4%)	1.15 (0.93, 1.41)	0.197
Water treated with chlorine	221 (48.0%)	860 (44.7%)	1.15 (0.94, 1.41)	0.184
c) Health at 60d follow-up				
Diarrhea	201 (44.2%)	772 (40.4%)	1.08 (0.88, 1.33)	0.465
Visited health facility for diarrhea in last 60 day	77 (16.7%)	277 (14.4%)	1.08 (0.81, 1.41)	0.608
Dysentery in last 60d	5 (1.1%)	19 (1.0%)	1.16 (0.38,2.92)	0.772
Visited health facility for dysentery in last 60 day	1 (0.2%)	11 (0.6%)	0.37 (0.02, 1.92)	0.341
Fever in last 60 day	254 (55.8%)	1097 (57.3%)	0.92 (0.75, 1.13)	0.416
Visited health facility for fever in last 60day	79 (17.2%)	364 (18.9%)	0.85 (0.65, 1.11)	0.243
Death of child	6 (1.3%)	7 (0.4%)	**3.20 (1.02, 9.72)**	**0.038**

[1] Any pathogens detected in a child's stool specimen at enrollment that were significantly associated with moderate-to-severe diarrhea (MSD) at the GEMS Kenya site [5].

[2] All adjusted ORs (aORs) adjusted for age group and sex of control child.

[3] Water source that does not meet the criteria for "improved," per the Joint Monitoring Program criteria [40] of a source that is safely protected from outside contamination (especially feces) via its construction or intervention

[4] Effective water treatment classified as solar disinfection, chlorine disinfection, boiling, or filtration through ceramic or other filter. Ineffective water treatment classified as filtration through a cloth, alum, or other chemical added

Table 4:

Differences in health and WASH conditions among controls by MSD pathogen detection in stool and reported diarrhea, Global Enteric Multicenter Study, Kenya site[1]

Parameter	Gl + G3[2] Diarrhea aOR (95% CI)[3] n = 919	G2 No diarrhea, ≥1 MSD pathogens[3] detected aOR (95% CI)[4] n = 262	G4 No diarrhea, 0 MSD pathogens[3] detected aOR (95% CI)[4] n = 1,203
Health at enrollment			
Fever (in last 7 days)	**1.68 (1.40, 2.01)**	1.20 (0.90, 1.58)	Ref.
Vomiting (in last 7 days)	**1.74 (1.03, 2.99)**	1.96 (0.91, 3.96)	Ref.
WASH conditions at enrollment			
Any sanitation facility present	1.09 (0.90, 1.34)	1.36 (0.99, 1.91)	Ref.
Unimproved water source[5]			Ref.
0–11 mo	1.12 (0.83, 1.50)	0.96 (0.61, 1.50)	
12–23 mo	1.16 (0.85, 1.58)	**1.95 (1.22,3.11)**	
24–59 mo	0.93 (0.67, 1.30)	0.58 (0.33, 1.03)	
Water treated	1.04 (0.87, 1.23)	1.22 (0.93, 1.61)	Ref.
Water treated effectively[6]			Ref.
0–11 mo	1.07 (0.76, 1.52)	0.85 (0.50, 1.42)	
12–23 mo	1.00 (0.70, 1.42)	**1.94 (1.09, 3.45)**	
24–59 mo	1.21 (0.83, 1.78)	1.15 (0.62, 2.14)	
Water treated with chlorine			Ref.
0–11 mo	1.27 (0.90, 1.79)	1.02(0.60, 1.72)	
12–23 mo	1.07 (0.75, 1.53)	**1.93(1.11,3.37)**	
24–59 mo	1.24 (0.85, 1.82)	1.23 (0.66, 2.28)	
Health at 60-day follow-up			
Visited health facility for diarrhea	**4.77 (3.67, 6.27)**	0.84 (0.47, 1.41)	Ref.
Dysentery	**3.77 (1.53, 10.6)**	1.62 (0.24, 7.09)	Ref.
Visited health facility for dysentery	-	-	
Fever	**1.77 (1.48, 2.12)**	0.85 (0.65, 1.12)	Ref.
Visited health facility for fever	**1.46 (1.17, 1.82)**	0.81 (0.54, 1.17)	Ref.

Bold indicates significant at 0.05.

[1]Multivariable logistic regression models used for all;

[2]G1 and G3 were combined to represent all control children for whom diarrhea was reported in the 14 days following enrollment;

[3]Any pathogens detected in a child's stool specimen at enrollment that were significantly associated with moderate-to-severe diarrhea (MSD) at the GEMS Kenya site [5].

[4]Adjusted for age group and sex, stratified estimates by age group and/or sex presented where effect modification significant at 0.05 was observed;

[5]Water source that does not meet the criteria for "improved," per the Joint Monitoring Program criteria [40] of a source that is safely protected from outside contamination (especially feces) via its construction or intervention;

[6]Effective water treatment classified as solar disinfection, chlorine disinfection, boiling, or filtration through ceramic or other filter. Ineffective water treatment classified as filtration through a cloth, alum, or other chemical added.

Table 5:

Odds Ratios for stunting among controls by diarrhea and enteric pathogen detection in stool, Global Enteric Multicenter Study, Kenya site[1]

a) Reported diarrhea		
Diarrhea **+/−**	Any diarrhea reported n = 919	No diarrhea reported n = 1,465
Enrollment	1.10 (0.92, 1.33)	Ref.
60-day follow-up	0.99 (0.73, 1.34)	Ref.
b) Pathogen detection in stool		
MSD pathogen [2] **+/−**	≥1 MSD pathogens detected n = 460	0 MSD pathogens detected n = 1,924
Enrollment	1.12 (0.89, 1.41)	Ref.
60-day follow-up	**1.57 (1.09, 2.23)**	Ref.
Any potential enteric pathogen [3] **+/−**	1+ potential pathogens detected n = 1,629	0 enteric pathogens detected n = 755
Enrollment	**1.29 (1.06, 1.57)**	Ref.
60-day follow-up	1.14 (0.84, 1.57)	Ref.

c) Diarrhea/MSD enteric pathogen groups (G1–4)			
Diarrhea and MSD enteric pathogen **+/−**	G1 + G3[4] Diarrhea n = 919	G2 No diarrhea, 1+ MSD pathogen[2] detected n = 262	G4 No diarrhea, 0 MSD pathogens[2] detected n = 1,203
Enrollment	1.11 (0.91, 1.34)	1.01 (0.74, 1.36)	Ref.
60-day follow-up	1.04 (0.76, 1.43)	1.31 (0.81, 2.08)	Ref.

[1] Multivariable logistic regression models used for stunting outcomes. All models are adjusted for age group and sex. Models at 60-day follow-up include stunting at baseline as a predictor as well. **Bold** indicates significance at 0.05 level.

[2] Any pathogens detected in a child's stool specimen at enrollment that were significantly associated with moderate-to-severe diarrhea (MSD) at the GEMS Kenya site [5].

[3] Any pathogens detected from the entire list of potential pathogens assessed in GEMS [22]

[4] G1 and G3 were combined to represent all control children for whom diarrhea was reported in the 14 days following enrollment