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Pathogen-Specific Burden of Outpatient Diarrhea in Infants in Nepal: A Multisite Prospective Case-Control Study

Cristina V. Cardemil[1], **Jeevan B. Sherchand**[2], **Laxman Shrestha**[2], **Arun Sharma**[2], **Howard E. Gary Jr.**[1], **Concepcion F. Estivariz**[1], **Marta Diez-Valcarce**[3], **M. Leanne Ward**[3], **Michael D. Bowen**[3], **Jan Vinjé**[3], **Umesh Parashar**[3], **Susan Y. Chu**[1]

[1]Global Immunization Division, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia

[2]Institute of Medicine, Tribhuvan University, Kathmandu, Nepal

[3]Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract

Background.—Nonsevere diarrheal disease in Nepal represents a large burden of illness. Identification of the specific disease-causing pathogens will help target the appropriate control measures.

Methods.—Infants aged 6 weeks to 12 months were recruited from 5 health facilities in eastern, central, and western Nepal between August 2012 and August 2013. The diarrhea arm included infants with mild or moderate diarrhea treatable in an outpatient setting; the nondiarrhea arm included healthy infants who presented for immunization visits or had a mild nondiarrheal illness. Stool samples were tested for 15 pathogens with a multiplex polymerase chain reaction (PCR) assay and real-time reverse-transcription (RT)-PCR assays for rotavirus and norovirus. Rotavirus- and norovirus-positive specimens were genotyped. We calculated attributable fractions (AFs) to estimate the pathogen-specific burden of diarrhea and adjusted for facility, age, stunting, wasting, and presence of other pathogens.

Results.—We tested 307 diarrheal and 358 nondiarrheal specimens. Pathogens were detected more commonly in diarrheal specimens (164 of 307 [53.4%]) than in nondiarrheal specimens (113 of 358 [31.6%]) ($P < .001$). Rotavirus (AF, 23.9% [95% confidence interval (CI), 14.9%–32.8%]), *Salmonella* (AF, 12.4% [95% CI, 6.6%–17.8%]), and *Campylobacter* (AF, 5.6% [95% CI, 1.3%–9.8%]) contributed most to the burden of disease. In these diarrheal specimens, the most common

Correspondence: C. V. Cardemil, MD, MPH, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Atlanta, GA 30333 (iyk8@cdc.gov).

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

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genotypes for rotavirus were G12P[6] (27 of 82 [32.9%]) and G1P[8] (16 of 82 [19.5%]) and for norovirus were GII.4 Sydney (9 of 26 [34.6%]) and GII.7 (5 of 26 [19.2%]).

Conclusions.—The results of this study indicate that the introduction of a rotavirus vaccine in Nepal will likely decrease outpatient diarrheal disease burden in infants younger than 1 year, but interventions to detect and target other pathogens, such as *Salmonella* and *Campylobacter* spp, should also be considered.

Keywords

Campylobacter; diarrhea; Nepal; norovirus; rotavirus; *Salmonella*

Globally, diarrhea is among the top 3 causes of death in children younger than 5 years [1, 2]. In Nepal, diarrhea has been estimated to account for 15% of deaths in 1- to 59-month-old children [3], and up to 24% of caretakers of infants younger than 24 months reported that the infants had diarrhea in the 2 weeks preceding the national Demographic and Health Survey [4].

Data on the etiologies of diarrheal disease in developing countries are scarce [5, 6], yet they can help to inform policies and interventions. In Nepal, studies on the etiology of diarrhea have found high rates of parasitosis, bacteria, and/or viruses [7-13], depending on the age group and setting studied. Rotavirus is the most common pathogen in children younger than 5 years of age who are hospitalized with acute gastroenteritis, and it is responsible for 26% to 39% of diarrheal cases [9, 11, 14-16]. This high burden of rotavirus gastroenteritis drove the Ministry of Health of Nepal to plan the introduction of a rotavirus vaccine in 2016–2018.

Although past studies have contributed greatly to our understanding of the pathogens associated with diarrhea in Nepal, knowledge gaps that are important for targeting effective prevention measures still exist. Many studies focused on 1 pathogen or time of year or on hospitalized patients, who tend to have moderate-to-severe diarrhea [7, 9, 11, 12, 14, 15, 17-20], but few studies have examined less severe diarrhea, which is responsible for a large burden of illness in Nepal and other developing countries and might be caused by different pathogens [6]. Few studies included a control group, which can aid in differentiating causality versus colonization or shedding without disease but increase the cost of the study [7, 17]. In addition, laboratory methods that are needed to detect diverse pathogens have evolved rapidly, which makes individual testing for each pathogen time and resource intensive. Recently developed polymerase chain reaction (PCR)-based tests for simultaneously detecting multiple pathogens with high sensitivity and specificity are now commercially available [21].

As part of a larger study [22], stool samples from infants with and those without mild-to-moderate diarrhea at 5 outpatient centers in western, central, and eastern Nepal throughout a 1-year period were tested for multiple enteric pathogens. We used a PCR-based multiplex panel to detect 15 common pathogens, and we conducted genotyping for rotavirus- and norovirus-positive samples. Our objectives were to estimate the pathogen-specific burden of diarrhea in these infants and determine the most common circulating rotavirus and norovirus genotypes.

METHODS

Study Procedures

Between August 2012 and August 2013, we recruited infants aged 6 weeks to 12 months who presented to the outpatient clinic or emergency department of 5 study sites throughout Nepal (Kosi Zonal Hospital, Biratnagar, and B.P. Koirala Institute of Health Sciences, Dharan [eastern Nepal], Kanti Children's Hospital, Kathmandu [central Nepal], and Western Regional Hospital, Pokhara, and Nepalgunj Medical College, Nepalgunj [western Nepal]).

Infants in the diarrhea arm had 3 or more loose stools in the 24 hours before study entry. Infants assigned to the non-diarrheal/control arm included those who were healthy and attending immunization visits and those who presented with a mild acute nondiarrheal illness. To decrease potential detection of pathogens from convalescent individuals, control infants were excluded if they had diarrhea in the 2 weeks before study enrollment. Infants with clinical illness that required hospital admission (such as severe dehydration) or those with blood in the stool were excluded from participation; therefore, the infants with diarrhea who remained in the study were those with mild-to-moderate diarrhea who were treated on an outpatient basis. Because this study was part of a larger study in which the effect of diarrheal disease on oral polio vaccine (OPV) was being examined, infants were excluded if they had received >2 previous OPV doses [22].

After obtaining written informed consent from the caregiver, a study physician conducted an interview with the infant's caregiver to gather information on demographics, clinical history, and treatment. Then, each infant had his or her temperature taken and was weighed and measured, and a stool sample was collected.

Laboratory Testing

Stool samples were placed at 2 to 8°C within 6 hours after collection, stored at −20°C, and shipped to the Centers for Disease Control and Prevention laboratory for testing. Samples were tested using an xTAG gastrointestinal pathogen panel (GPP) (Luminex Molecular Diagnostics, Toronto, Ontario, Canada) to simultaneously screen for 15 enteric pathogens. Samples positive for norovirus according to the GPP were confirmed by real-time reverse-transcription (RT)-PCR [23]. Samples positive according to the GPP for rotavirus were genotyped directly, whereas samples that were negative according to the GPP for rotavirus were tested by RT-PCR [24, 25]. Rotavirus- and norovirus-positive samples were genotyped using methods described previously [23, 24]. For rotavirus, participants were classified as positive if either the GPP or RT-PCR result was positive; for norovirus, participants were classified as positive or negative according to the real-time RT-PCR result.

Statistical Analyses

The percentages of infants with pathogens identified in the diarrhea and nondiarrhea arms were determined using standard binomial proportions with 95% Wilson score confidence intervals (CIs), and pathogens between the 2 arms were compared using the 2-tailed Fisher exact test. Because samples from all norovirus-positive participants were tested by real-time

RT-PCR, cycle threshold (Ct) values between the diarrhea and nondiarrhea arms were compared by using the Wilcoxon-Mann-Whitney test.

For the pathogens that differed significantly between the diarrhea and nondiarrhea arms, we calculated adjusted attributable fractions (AFs) to estimate the pathogen-specific burden of diarrhea [26] after adjusting for facility, age, stunting, wasting, and the other pathogens. The AF is an estimate of the percentage of diarrhea cases that can be attributed to infection with the pathogen of interest. The AFs were calculated using adjusted odds ratios as determined by logistic regression. CIs for the AFs were calculated using the bootstrap procedure with 1000 replicates.

Vesikari score components were determined according to standard practice [27], with the exception of temperature; because the patients were not hospitalized, their temperature at study entry was recorded. Stunting and wasting were determined by calculating length-for-age and weight-for-length *z* scores, respectively, from the World Health Organization child growth standards [28]. Data were double-entered into an Access (Microsoft, Redmond, WA) database and analyzed using SAS 9.3 (SAS Institute, Cary, NC) and R 3.3.2 and the R package attribrisk (R Foundation for Statistical Computing, Vienna, Austria).

Ethical Review

This study underwent ethical review and received approval from the Tribhuvan University Institute of Medicine, the Nepal Health Research Council, and the US Centers for Disease Control and Prevention.

RESULTS

We enrolled 324 participants with diarrhea and 375 participants without diarrhea; 3 (1%) of those with diarrhea were excluded because they did not meet the eligibility criteria for the study. An additional 14 (4%) participants with diarrhea and 17 (4%) without diarrhea did not have samples available for testing, which left 307 participants in the diarrhea arm and 358 in the nondiarrhea arm. Participants in the diarrhea arm and those in the nondiarrhea arm differed slightly according to age group, study facility, lifetime previous episodes of diarrhea, and stunting ($P < .05$ for each of these characteristics) (Table 1).

Among those in the diarrhea arm, 1 or more pathogens were identified in 53% (164 of 307), whereas among those in the nondiarrhea arm, 1 or more pathogens were identified in 32% (113 of 358) ($P < .001$). Of the 164 participants with diarrhea and any infection identified, 51% (86) had a single pathogen identified, 34% (55) were coinfected with 2 pathogens, and 16% (26) were coinfected with 3 or more pathogens (Supplementary Table 1). Of the 113 participants in the nondiarrhea arm with any infection identified, a single pathogen was in identified 72% (81), 21% (24) were coinfected with 2 pathogens, and 7% (8) were coinfected with 3 or more pathogens.

Participants in the diarrhea arm and those in the nondiarrhea arm were more likely to be infected with a virus (43% vs 20%, respectively; $P < .001$) or bacteria (31% vs 13%, respectively; $P < .001$) than with a parasite (7% vs 4%, respectively; $P = .085$) (Table 2).

Overall, rotavirus was the most common pathogen identified, and it was detected more frequently in the diarrhea arm than in the nondiarrhea arm (37% vs 15%, respectively; $P <$ .001). Other pathogens more commonly detected in the diarrhea arm than in the nondiarrhea arm included *Salmonella* (17% vs 4%, respectively; $P <$ .001) and *Campylobacter jejuni* (10% vs 4%, respectively; $P =$ .004). Other common enteric pathogens that did not differ significantly between the diarrhea and nondiarrhea arms included norovirus (8% vs 6%, respectively; $P =$ .372), *Cryptosporidium* (5% vs 2%, respectively; $P =$ .127), and *Clostridium difficile* (4% vs 4%, respectively; $P =$ .846).

Overall, 32% of diarrheal episodes could be attributed to known enteric pathogens. Viral infections had the highest AF (26.9% [95% CI, 17.2%–35.3%]), followed by bacterial (19.7% [95% CI, 12.3%–26.6%]) and parasitic (1.1% [95% CI, −3.9% to 5.5%]) infections. For individual pathogens, the highest AFs were found for rotavirus (23.9% [95% CI, 14.9–32.8]), followed by *Salmonella* (12.4% [95% CI, 6.6%–17.8%]) and *Campylobacter* (5.6% [95% CI, 1.3%–9.8%]).

In the diarrhea arm, the most common rotavirus genotypes identified were G12P[6] (27 of 82 [33%]) and G1P[8] (16 of 82 [20%]); the most common norovirus genotypes were GII.4 Sydney (9 of 26 [35%]) and GII.7 (5 of 26 [19%]) (Table 3). Between norovirus-positive participants in the diarrhea arm and those in the nondiarrhea arm, no statistically significant differences in the distributions of the Ct values were found (median [range], 31.2 [18.2–38.8] vs 27.6 [16.8–38.4], respectively; $P =$ .3).

Pathogens in participants with diarrhea were identified more commonly in infants aged 9 through 12 months (Figure 1) and between December and June (Figure 2 and Supplementary Table 2). Rotavirus infection was detected throughout the year but was predominant from December through June, whereas norovirus infection was predominant from March through June and *Salmonella* was predominant from February through May.

The median duration of diarrhea was 6 days (interquartile range, 4–9), and the median of the maximum number of stools per day was 6 (interquartile range, 5–6) (Table 4). Fever was a more common symptom for rotavirus-positive participants, whereas nausea and vomiting were reported more often for those who tested positive for norovirus. Participants with diarrhea and *Salmonella* or *Campylobacter* were not readily distinguishable by any 1 clinical symptom.

Before study entry, 47% (145) of participants with diarrhea were reported to have received some form of treatment. Of those for whom treatment was reported, 56% were treated at a clinic, and 48% were treated at a hospital. Of the infants with diarrhea (n = 307), fewer than half had received oral rehydration salts (ORS) (37%) or zinc (28%).

Overall, receipt of an antibiotic before study entry was reported for 15% of participants in the diarrhea arm, and the percentage of those who received any antibiotic did not differ markedly according to identified-pathogen group (9.2% viral, 9.1% parasitic, and 11.7% bacterial) (Table 5). Reported antibiotic use did not differ for most participants with diarrhea and a specific infection, with the exception of rotavirus; the percentage of rotavirus-positive

participants for whom antibiotic use was reported (23.9%) was lower than the percentage of those for whom no antibiotic use was reported (39.8%) ($P = .047$).

DISCUSSION

In this multisite outpatient case-control study of mild-to-moderate diarrhea in Nepal, the pathogen-specific burden of diarrhea was attributable largely to rotavirus, followed by *Salmonella* and *Campylobacter*. We detected a high number and diverse range of pathogens in both the diarrhea and nondiarrhea arms, with a predilection for older infants (9–12 months) and the winter and spring months, along with pathogen-specific variations in seasonality.

Rotavirus is known to be an important pathogen in children hospitalized with acute gastroenteritis in Nepal [9, 11, 12, 14-19]. In 2 previous studies that examined diarrheal pathogens among Nepalese outpatients [11] and rural settings [17], the prevalence of rotavirus in stools was lower than that in our study (4.1% in a rural village and 16.8% in an outpatient population vs 37% in our group of outpatients with diarrhea).

We also found a higher background rate of rotavirus-positive samples in our nondiarrhea arm (15%) than had been detected in 2 previous studies (range, 0.9%–2%) [7, 17]. Increased sensitivity of the real-time RT-PCR assay over that of the enzyme-linked immunosorbent assay and/or detection of a past illness because rotavirus can be shed for weeks [29, 30] might partially explain our results. Nevertheless, the presence of rotavirus in participants of both arms of our study, the higher rate of infection in the older infants in our sample, and increased detection rates in the winter months are consistent with past study results. These data support the Ministry of Health's plans to introduce a rotavirus vaccine to the routine immunization schedule of Nepal. The most frequent genotypes detected in our study, G12P[6] and G1P[8], are also consistent with those previously identified in surveillance of hospitalized children with acute gastroenteritis [14-16, 18, 19]. Because both of the currently available rotavirus vaccines have been found to be protective against homotypic and heterotypic strains, the vaccine is expected to have a large impact in decreasing the burden of rotavirus infection in infants and children in Nepal [31]. Continuing surveillance before and after introduction of the rotavirus vaccine will be important for quantifying the degree of strain-specific vaccine effectiveness and for monitoring the emergence of new strains.

Norovirus has been studied less frequently as a diarrheal pathogen in Nepal. The MAL-ED study, a prospective longitudinal study conducted at 8 sites worldwide, including Bhaktapur, Nepal, found norovirus GII to have a high attributable burden of diarrhea, especially in children in their second year of life [6]. Hoa-Tran et al. [20] detected norovirus in 8% of children <5 years of age who were hospitalized with acute gastroenteritis in Kanti Children's Hospital in Kathmandu between 2005 and 2011 and predominantly in the 6- to 23-month age group. GII.4 was the most common genotype identified in their study, followed by GII.3 and GII.13, and more norovirus cases were detected from September through December. We also found that GII.4 Sydney was the most common genotype, and older infants had a higher frequency of norovirus detection; however, we detected norovirus

most commonly from March through June. It is also notable that we found no statistical difference in the percentages of norovirus-positive samples in the diarrhea and nondiarrhea arms, which indicates that asymptomatic children were likely not able to clear a previous norovirus infection. Despite the advantages of case-control studies, the interpretation of results for norovirus and other infections that frequently result in asymptomatic excretion is challenging, because RT-PCR assays are extremely sensitive and can detect loads as low as 10^5 copies per g of stool [32]. In volunteers experimentally infected with norovirus, peak titers were detected in stool samples collected after resolution of symptoms, and the timings of onset, peak, and resolution of shedding were similar for inoculated participants regardless of whether they developed clinical gastroenteritis [33]. This fact might explain our results indicating no statistically significant difference in Ct values between norovirus-positive participants in the diarrhea arm and those in the nondiarrhea arm. Given the importance of norovirus as a major cause of diarrheal disease globally [6, 34], more studies are needed, including in Nepalese children older than 12 months, to better understand the role of norovirus infection in this population.

Salmonella spp had the second highest AFs in our study. Nontyphoidal *Salmonella enterica* and *Salmonella* Typhi are known causes of diarrheal disease in the southeast Asia region [34]. Two recent large multicountry case-control diarrheal etiology studies identified *Salmonella* spp in their populations, but it was not a very common pathogen [5, 6]. Similarly, studies in Nepal also identified *Salmonella* spp in <4% of children hospitalized with acute gastroenteritis [6, 10, 13]. It is possible that previous antibiotic exposure can result in non-viable organisms that limit the detection of certain pathogens in culture, which was the method used in these studies, but PCR-based methods that detect remaining nucleic acid fragments might result in a higher detection rate [35]. In Nepal, antibiotics can be bought over the counter, and paramedical health workers tend to prescribe antibiotics to their patients; indeed, in this study, receipt of antibiotics before stool testing was reported for 11.5% of participants with *Salmonella* infection in the diarrhea arm. Because the Luminex GPP is a PCR-based assay, it is likely to be more sensitive for some bacteria than traditional culture. The Luminex GPP assay was found to have high sensitivity and specificity for detecting 15 pathogens, including *Salmonella* spp [21], although the results of another study suggested that confirmatory testing might be needed for *Salmonella* and *Entamoeba histolytica* [36]. In our study, the high AF for *Salmonella* and its infrequent detection in the nondiarrhea arm suggest that infants with positive results for *Salmonella* according to the Luminex GPP were true positives. Given that infants are more likely to have severe illness that results from *Salmonella* infection and are at higher risk for complications from diarrheal disease [37], additional studies to confirm this finding and determine the *Salmonella* serovars most associated with illness in this age group are important for improving diagnosis and treatment.

Campylobacter also was detected frequently in our population, and that frequency increased with age. Globally, *Campylobacter* was responsible for an estimated 96 million foodborne cases and more than 21 000 deaths in 2010 [34]. It has been identified in Nepalese children with diarrhea [6, 7, 38], although in 1 study, it was identified only in children aged 12 to 24 months [6]. As with other bacterial pathogens, detection by culture might result in an underestimate of *Campylobacter* burden compared with enzyme-linked immunosorbent

assay or PCR-based methods [6, 35]; Luminex detected most *Campylobacter* positives when compared to real-time PCR and culture [21, 36].

Collecting data on symptoms and treatment before study entry enabled us to examine differences in clinical presentation and antibiotic use by pathogen. Overall, antibiotic use before study entry was reported for 15% of participants in the diarrhea arm. Although it is encouraging that antibiotic use was reported for fewer rotavirus-positive participants, the reason for less-frequent antibiotic use for this virus but not for other pathogens is not readily apparent. One possible explanation is that rotavirus illness often starts with vomiting and is followed by diarrhea, and because international diarrhea-management guidelines for children generally limit antibiotic use to very specific situations [39], practitioners might have classified the illness as viral on the basis of clinical presentation. Alternatively, if practitioners are aware of the predominantly winter seasonality of rotavirus, as opposed to that of other pathogens, their practice patterns and treatment recommendations might differ throughout the calendar year.

Compared with antibiotic use, other forms of treatment were more common but still fell short of national and international guidelines. Fewer than half of the diarrhea arm participants had received ORS, and only 28% had received zinc, despite being recommended by the World Health Organization and in Nepal to reduce the severity and duration of illness and reduce the number of future diarrheal episodes [40]. Non-pathogen-specific interventions for diarrhea such as ORS and zinc are particularly useful in a setting such as Nepal, where the rates of diarrheal disease, stunting, and zinc deficiency are high, and diarrhea can have multiple etiologies; an infectious etiology was not identified for half of the participants in the diarrhea arm of our study.

This study had limitations. First, we purposefully included infants who were underimmunized for OPV, because this study was conducted as part of a larger one in which the effect of diarrhea on OPV seroconversion was examined, which might affect generalizability of the results. However, the demographics of our study population, including educational indicators, were similar to those surveyed in the Demographic and Health Survey, except our population was slightly more urban [4]. Second, although our study population was large enough to detect many pathogens and genotypes for rotavirus and norovirus, small sample sizes of those with some pathogens might have limited our ability to understand their role in causing diarrhea in this population. These pathogens include adenovirus and enterotoxigenic *Escherichia coli*, which have been identified as pathogens associated with diarrhea globally and in Nepal [5, 6]. Third, although the Luminex GPP tests for 15 pathogens, it does not detect parasites identified previously in Nepal, such as *Ascaris, Trichuris,* and *Cyclospora* spp [7, 12, 41, 42]. However, these parasites are known to result in a higher burden of disease in older, school-aged children, and we examined samples for parasites in Kathmandu and identified few to none of these pathogens (data not shown).

This study also had several strengths. First, addressing the etiology of mild-to-moderate diarrhea in an outpatient setting complements previous hospital-based studies, which were biased more toward severe disease. Second, including a control group enabled us to calculate the AFs and pathogen-specific burdens of disease and provided a more reliable test to assign

causality than in studies that rely only on cases. In addition, the pathogens identified in the control group contribute to our understanding of colonization versus shedding in these asymptomatic participants and warrant further study, such as in prospective longitudinal studies (eg, quantification of viral load to help us understand true pathogenicity and the role of these infections in other processes such as stunting, wasting, and immune responses). Third, we enrolled participants over 1 year from diverse backgrounds and living conditions from 5 study sites in western, central, and eastern Nepal, which increased the representativeness of our sample and enabled us to assess seasonality. Fourth, the Luminex GPP assay detected multiple pathogens in a single sample with high sensitivity and specificity. The use of such tests can be less expensive [35] and enable more comprehensive diarrhea etiology studies in settings in which it might not be possible otherwise.

In summary, our results show that rotavirus is responsible for a high proportion of nonsevere diarrheal disease, which complements the findings of other studies that revealed the crucial role of rotavirus in moderate-to-severe diarrhea. In addition, our results suggest that *Salmonella* and *Campylobacter* spp play an important role in nonsevere diarrhea in infants, and the role of norovirus needs to be investigated further. In addition to full implementation of known effective interventions, such as administering a rotavirus vaccine and providing zinc and ORS during diarrheal episodes, developing new ways to detect and target other common pathogens and addressing the large number of cases with unknown etiologies will help reduce the burden of diarrheal disease in Nepal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

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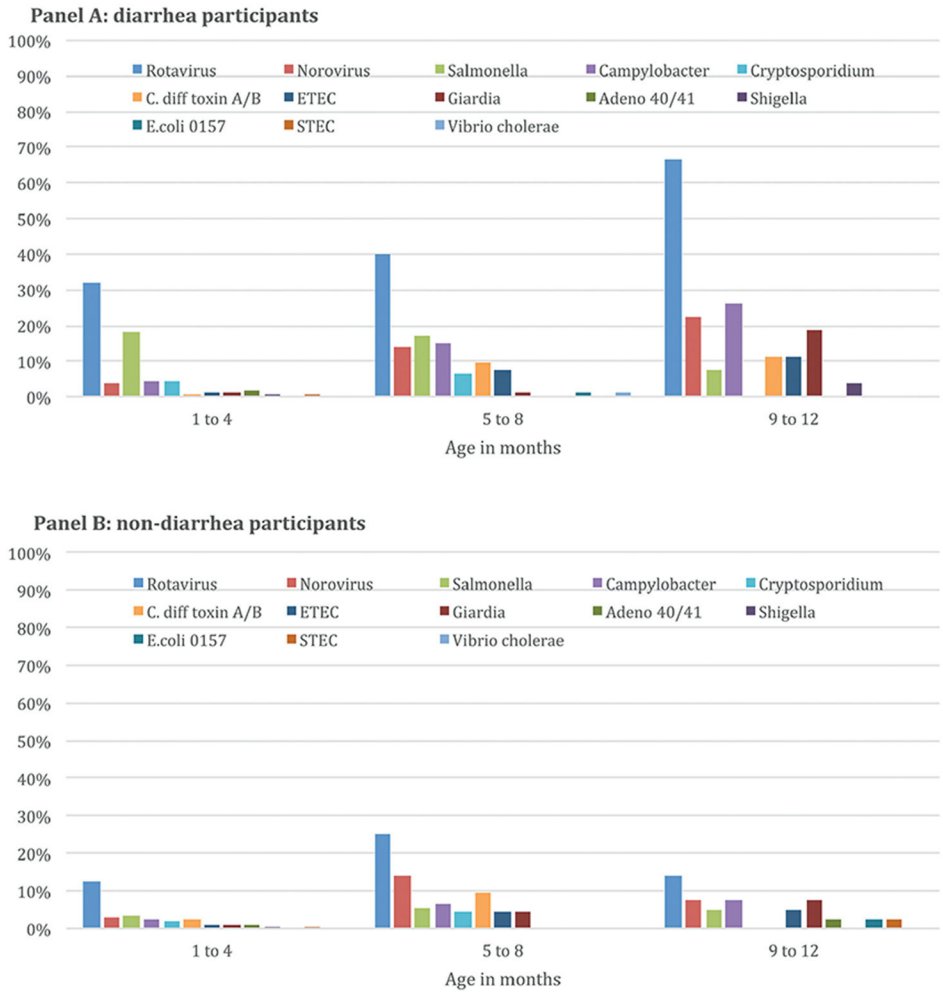

Figure 1.

Percentages of infections in the diarrhea (A) and nondiarrhea (B) arms, according to pathogen and age group. The numerator is number of persons in that age group whose sample tested positive for a specific infection; the denominator is number of persons in the age group. Abbreviations: Adeno, adenovirus; C.diff, *Clostridium difficile*; E.coli, *Escherichia coli*; ETEC, enterotoxigenic *E*coli LT/ST; Norovirus, Norovirus GI/GII; STEC, Shiga-like toxin producing *E coli* stx_1/stx_2.

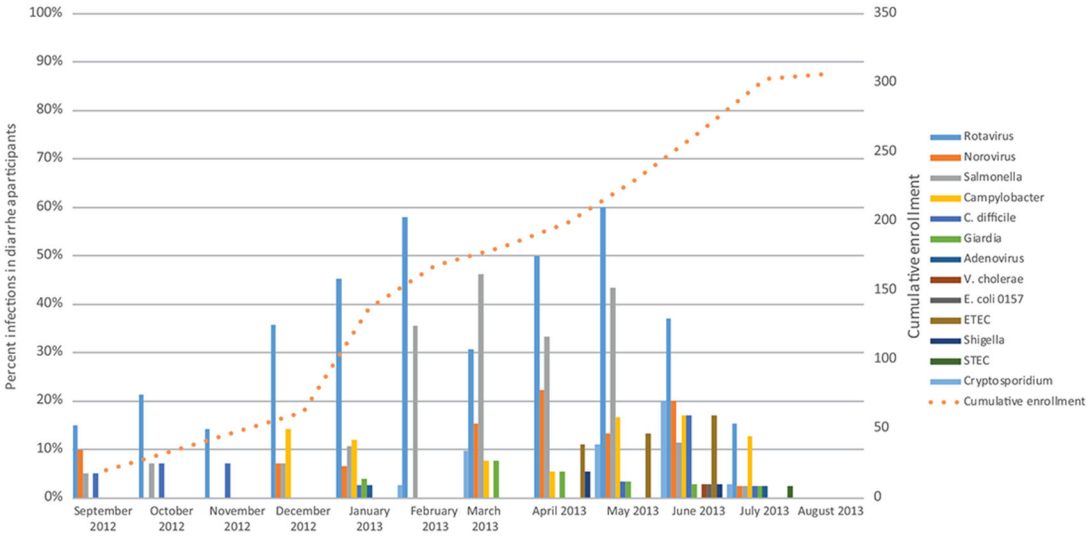

Figure 2.

Infections and cumulative enrollment in the diarrhea arm, according to month of the year (September 2012 through August 2013). The numerator is number of persons in that month whose sample tested positive for a specific infection; denominator is the number of persons enrolled in that month. Abbreviations: C. difficile, *Clostridium difficile*; E. coli, *Escherichia coli* LT/ST; ETEC, enterotoxigenic *E cofi*; Norovirus, Norovirus GI/GII; STEC, Shiga-Toxin producing *E coli* stx_1/stx_2; V. cholerae, *Vibrio cholerae.*

Table 1.

Characteristics of Participants in the Diarrhea and Nondiarrhea Arms

	Diarrhea Arm (N = 307)			Nondiarrhea Arm (N = 358)			
Characteristic	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**P[a]**
Facility							.002
Kanti	38	12	9–16	80	22	18–27	
Kosi Zonal	72	23	19–28	66	18	15–23	
Western Regional	47	15	12–20	36	10	7–14	
Nepalgunj Medical College	61	20	16–25	86	24	20–29	
BPKIHS	89	29	24–34	90	25	21–30	
Age							.009
6–12 wk	91	30	25–35	146	41	36–46	
13–25 wk	146	48	42–53	137	38	33–43	
26–51 wk	70	23	18–28	75	21	16–26	
Sex							.388
Male	169	55	50–60	210	59	54–64	
Female	138	45	40–50	148	41	36–46	
Religion							.050
Hindu	280	91	88–94	300	84	80–87	
Muslim	13	4	2–7	31	9	6–12	
Buddhist	9	3	2–6	16	4	3–7	
Christian	5	2	1–4	10	3	2–5	
Other	0	0	0–1	1	0	0–2	
Mother’s education							.689
No formal schooling	93	30	25–36	103	29	24–34	
Some primary	68	22	18–27	87	24	20–29	
Completed primary	27	9	6–12	23	6	4–10	
Some secondary	36	12	9–16	36	10	7–14	
Obtained SLC	49	16	12–20	60	17	13–21	
Higher education	34	11	8–15	49	14	10–18	
No. of previous diarrheal episodes							<.001

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Characteristic	Diarrhea Arm (N = 307) n	%	95% CI	Nondiarrhea Arm (N = 358) n	%	95% CI	P[a]
0	173	56	51–62	251	70	65–75	
1	50	16	13–21	47	13	10–17	
2	38	12	9–16	44	12	9–16	
3	17	6	4–9	9	2	1–5	
4	10	3	2–6	2	1	0–2	
≥5	19	6	4–9	5	1	1–3	
Residence							.172
Urban	106	34	29–40	143	40	35–45	
Rural	201	66	60–71	215	60	55–65	
Zone							.297
Mountain	17	6	4–9	16	4	3–7	
Hill	143	47	41–52	188	52	47–58	
Terai	147	48	42–54	154	43	38–48	
Stunting							.031
Severe	32	10	8–14	25	7	5–10	
Moderate	45	15	11–19	35	10	7–13	
Mild/none	230	75	70–79	298	83	79–87	
Wasting							.024
Severe	26	8	6–12	50	14	11–18	
Moderate	37	12	9–16	28	8	6–11	
Mild/none	244	80	75–84	280	78	74–82	
Breastfeeding							.230
Exclusive	251	82	77–86	279	78	73–82	
Some	55	18	14–23	72	20	16–25	
None	1	0	0–2	5	1	1–3	
Don’t know	0	0	0–1	2	1	0–2	

Abbreviations: BPKIHS, B.P Koirala Institute of Health Sciences; CI, confidence interval; SLC, school leaving certificate.

[a]The *P* value shown is for the comparison of differences in proportions between participants in the diarrhea arm and those in the nondiarrhea arm using the 2-tailed Fisher exact test.

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Table 2.

Enteric Infections Identified in the Diarrhea and Nondiarrhea Arms

	Diarrhea Arm (N = 307)		Nondiarrhea Arm (N = 358)		
Infection Type	**% (n)**	**95% CI**	**% (n)**	**95% CI**	***P***
Viral	43 (131)[a]	37–48	20 (73)	16–25	<.001
Rotavirus[b]	37 (115)	32–43	15 (55)	12–19	<.001
Norovirus GI/GII[b]	8 (26)	6–12	6 (23)	4–9	.372
Adenovirus 40/41	1 (3)	0–3	1 (3)	0–2	1.000
Bacterial	31 (94)	26–36	13 (47)	10–17	<.001
Salmonella	17 (52)	13–22	4 (15)	3–7	<.001
Campylobacter jejuni	10 (29)	7–13	4 (14)	2–6	.004
Clostridium difficile toxin A/B	4 (13)	2–7	4 (14)	2–6	.846
Shigella	1 (2)	0–2	0 (1)	0–2	.598
Vibrio cholera	0 (1)	0–2	0 (0)	0–1	.462
Escherichia coli 0157	0 (1)	0–2	0 (1)	0–2	1.000
STEC stx_1/stx_2	0 (1)	0–2	1 (2)	0–2	1.000
ETEC LT/ST	4 (12)	2–7	2 (8)	1–4	.257
Yersinia enterocolitica	0 (0)	—	0 (0)	—	—
Parasitic	7 (22)	5–11	4 (14)	2–6	.085
Cryptosporidium	5 (14)	3–8	2 (8)	1–4	.127
Giardia	3 (8)	1–5	2 (9)	1–5	1.000
Entamoeba histolytica	0 (0)	—	0 (0)	—	—

Abbreviations: CI, confidence interval; ETEC, enterotoxigenic *Escherichia coli*; STEC, Shiga-like toxin-producing *Escherichia coli*.

[a]Because of coinfections, the numbers in the main infection-type headings (viral, bacterial, and parasitic) do not equal the sums of those in the subheadings. For the main headings, we used an indicator of infection by at least 1 of the pathogens in the group.

[b]Rotavirus results were obtained by the Luminex gastrointestinal pathogen panel (GPP) assay or reverse-transcription polymerase chain reaction (RT-PCR), and norovirus results were obtained by real-time RT-PCR. The remainder of pathogens were identified as positive by the Luminex GPP assay.

Table 3.

Genotyping of Rotavirus and Norovirus Infections in the Diarrhea and Nondiarrhea Arms

Genotype	Diarrhea Arm (n)	Nondiarrhea Arm (n)
Rotavirus	82	37
G1G2P[6]P[8]	1	—
G1G2P[8]	1	—
G1P[6]	1	—
G1P[6]P[8]	1	—
G1P[8]	16	1
G2P[4]	—	3
G2P[6]	1	—
G2P[8]	1	—
G8P[6]	—	1
G9P[6]	1	—
G9P[8]	1	—
G12P[6]	27	19
G12P[8]	1	—
GntP[6]	1	1
GntP[8]	2	—
GntP[nt][b]	27	12
Norovirus	26	23
GI		
GI.3	1[a]	1
GI.3D	—	2
GI.5	1	1
GI.6	2	—
GI.7	1	—
GII		
GII.2	1	—
GII.3	2	1
GII.4 Sydney	9[a]	9
GII.6	1	1
GII.7	5	—
GII.12	1	—
GII.13	—	1
GII.14	—	1
GII.17	—	3
GII.22	1	—
No sequence	2	3

[a]One participant in the diarrhea arm was coinfected with GI.3 and GII.4_Sydney.

[b]Of the rotavirus-positive specimens, 33% were nontypeable (GntP[nt]). Samples with a high cycle threshold (Ct) value contained too little genetic material to amplify for sequencing by conventional reverse-transcription polymerase chain reaction, and the Ct values for GntP[nt] specimens were higher (mean Ct, 34.4) than those of the genotyped specimens (mean Ct, 23.4).

Table 4.

Clinical Symptoms for Participants in the Diarrhea Arm at Study Entry

Symptom	All Participants (N = 307)	Rotavirus Positive[a] (N = 53)	Norovirus Positive[a] (N = 10)	*Salmonella* Positive[a] (N = 17)	*Campylobacter Positive*[a] (N = 10)
Watery stools (% [n])	92 (283)	89 (47)	100 (10)	100 (17)	90 (9)
Mucous in stools (% [n])	41 (127)	0 (0)	0 (0)	0 (0)	0 (0)
Maximum no. of loose stools per day					
Mean	5.9	5.9	6.5	4.6	5.2
Median	6	5	6.0	5	5.0
Range	3–14	3–14	5–10	3–6	3–8
IQR	5–6	5–6	6–7	4–5	4–6
No. of days with diarrhea					
Mean	8.4	6.1	7.0	5.4	6.3
Median	6	5	4	5	5.0
Range	4–76	1–28	1–28	3–13	2–16
IQR	4–9	4–6.5	4–6	4–6	4–8
Fever (% [n])	21 (64)	30 (16)	0 (0)	6 (1)	10 (1)
Nausea (% [n])[b]	8 (24)	4 (2)	30 (3)	0 (0)	10 (1)
Vomiting (% [n])	15 (47)	8 (4)	20 (2)	12 (2)	20 (2)
Maximum no. of vomiting episodes in 24 h					
Mean	4.6	5.8	*c*	*c*	*c*
Median	3.0	4	*c*	*c*	*c*
Range	1–25	1–25	*c*	*c*	*c*
IQR	2.5–6	2–4			
No. of days of vomiting					
Mean	2.6	1.8	*c*	*c*	*c*
Median	2	1	*c*	*c*	*c*
Range	0–20	0–5	*c*	*c*	*c*
IQR	1–3	0–3	*c*	*c*	*c*
Dehydration (% [n])[d]					
None	94 (290)	96 (51)	90 (9)	100 (17)	100 (10)

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Symptom	All Participants (N = 307)	Rotavirus Positive[a] (N = 53)	Norovirus Positive[a] (N = 10)	*Salmonella* Positive[a] (N = 17)	*Campylobacter Positive*[a] (N = 10)
Some	5 (16)	4 (2)	10 (1)	0 (0)	0 (0)
Vesikari score					
Mean	5.6	5.7	6.4	4.2	4.7
Median	5	5	6	4	4.0
Range	0–17	2–16	2–11	2–10	2–11
IQR	3–8	4–7	5–8	3–5	3–4
Wasting (% [n])					
Severe	8 (26)	8 (4)	30 (3)	6 (1)	0 (0)
Moderate	12 (37)	11 (6)	0 (0)	6 (1)	10 (1)
Mild/none	80 (244)	81 (43)	70 (7)	88 (15)	90 (9)
Stunting (% [n])					
Severe	10 (32)	8 (4)	10 (1)	0 (0)	10 (1)
Moderate	15 (45)	23 (12)	0 (0)	12 (2)	20 (2)
Mild/none	75 (230)	70 (37)	90 (9)	88 (15)	70 (7)

Abbreviation: IQR, interquartile range.

[a]Includes participants with diarrhea who had only the 1 pathogen listed; participants with coinfections were excluded.

[b]Because of the young age of the participants, when caregivers were asked if their infant had nausea, it was operationally translated as “Do you feel like your child had a tendency to vomit?”

[c]The number was too small (n = 2) to calculate meaningful statistics.

[d]Infants with severe clinical illness, including severe dehydration, were excluded from study participation.

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Table 5.

Antibiotic Use in Diarrhea Participants

	Number and percent receiving antibiotics, by infection type		Did Not Use Antibiotic (N=261)		Used Antibiotic (N=46)		
	n/N	Row %	N	Column %	N	Column %	*P*-value[a]
Viral	12/131	9.2%					
Adenovirus 40/41	0/3	0.0%	3	1.2	0	0.0	1.000
Norovirus GI/GII	4/26	15.4%	22	8.4	4	8.7	1.000
Rotavirus	11/115	9.6%	104	39.8	11	23.9	.047
Bacterial	11/94	11.7%					
C. difficile toxin A/B	1/13	7.7%	12	4.6	1	2.2	.700
Campylobacter	4/29	13.8%	25	9.6	4	8.7	1.000
ETEC LT/ST	1/12	8.3%	11	4.2	1	2.2	1.000
Salmonella	6/52	11.5%	46	17.6	6	13.0	.528
E. coli O157	0/1	0.0%	1	0.4	0	0.0	1.000
Shigella	0/2	0.0%	2	0.8	0	0.0	1.000
STEC stx1/stx2	0/1	0.0%	1	0.4	0	0.0	1.000
Cholera	0/1	0.0%	1	0.4	0	0.0	1.000
Parasitic	2/22	9.1%					
Cryptosporidium	1/14	7.1%	13	5.0	1	2.2	.702
Giardia	1/8	12.5%	7	2.7	1	2.2	1.000
Overall	46/307	15.0%					

[a] *p*-value represents Fisher's exact test for the difference in proportions of participants who did not use antibiotics with those who used antibiotics.