Journal of the Pediatric Infectious Diseases Society

ORIGINAL ARTICLE

Case-Control Assessment of the Roles of Noroviruses, Human Bocaviruses 2, 3, and 4, and Novel Polyomaviruses and Astroviruses in Acute Childhood Diarrhea

Rimma Melamed,[1,a] Gregory A. Storch,[1] Lori R. Holtz,[1] Eileen J. Klein,[2,4] Brittany Herrin,[1,b] Phillip I. Tarr,[1] and Donna M. Denno[2,4,3]

[1]Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri; Departments of [2]Pediatrics and [3]Global Health, University of Washington School of Medicine, Seattle, Washington; and [4]Department of Pediatrics, Seattle Children's Hospital, Seattle, Washington

Background. The etiology of acute childhood diarrhea often eludes identification. We used a case-control study-stool archive to determine if nucleic acid tests for established and newly identified viruses diminish our previously published 32% rate of microbiologically unexplained episodes.

Methods. Using polymerase chain reaction, we sought to detect noroviruses GI and GII, classic and novel astroviruses, and human bocaviruses (HBoVs) 2, 3, and 4 among 178 case and 178 matched control stool samples and St. Louis and Malawi polyomaviruses among a subset of 98 case and control stool samples. We calculated adjusted odds ratios and 95% confidence intervals using conditional logistic regression.

Results. Noroviruses were more common in cases (GI, 2.2%; GII, 16.9%) than in controls (GI, 0%; GII, 4.5%) (adjusted odds ratio, 5.2 [95% confidence interval, 2.5–11.3]). Astroviruses and HBoVs 2, 3, and 4 were overrepresented among the cases, although this difference was not statistically significant. Malawi polyomavirus was not associated with case status, and St. Louis polyomavirus was identified in only 1 subject (a control). When identified in cases, HBoVs 2, 3, and 4 were frequently (77%) found in conjunction with a bona fide diarrheagenic pathogen. Thirty-five (20%) case and 3 (2%) control stool samples contained more than 1 organism of interest. Overall, a bona fide or plausible pathogen was identified in 79% of the case stool samples. Preceding antibiotic use was more common among cases (adjusted odds ratio, 4.5 [95% confidence interval, 2.3–8.5]).

Conclusion. Noroviruses were found to cause one-third of the diarrhea cases that previously had no identified etiology. Future work should attempt to ascertain etiologic agents in the approximately one-fifth of cases without a plausible microbial cause, understand the significance of multiple agents in stools, and guide interpretation of nonculture diagnostics.

Keywords. antibiotics; children; diarrhea; human bocaviruses; viruses.

INTRODUCTION

Acute childhood diarrhea is common, but its microbial etiology often remains elusive. Polymerase chain reaction (PCR) assays offer us the opportunity to detect a greater diversity of enteric viruses in stool, including noroviruses GI and GII, human bocaviruses (HBoVs) 2, 3, and 4 [1, 2], and classic and newly described Melbourne (MLB) and Virginia (VA) clades of astrovirus [3]. This technology can be used also to elucidate the roles in diarrhea of 2 newly recognized polyomaviruses (PyVs) of humans, Malawi (MW) [4] and St. Louis (STL) [5] PyVs. Here, we report on an expanded analysis of a case-control study conducted among children who presented to the Seattle Children's Hospital (SCH) emergency department with acute diarrhea [6]. This work extends the findings of a predecessor study, in which we found that 68% of acute diarrhea episodes could be attributed to an identifiable definite or candidate pathogen and established a disease association with enteroaggregative *Escherichia coli* in acute childhood diarrhea in North America. In this study, we used PCR viral detection to more precisely define the role of viruses in community-acquired diarrhea.

Received 27 June 2016; editorial decision 26 November 2016; accepted 8 December 2016; published online February 10, 2017.

[a]Present Addresses: Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel; and Pediatric Infectious Diseases Unit, Soroka Medical Center, Beer Sheva, Israel.

[b]Present Address: A. T. Still University, Kirksville, Missouri.

Correspondence: D. M. Denno, MD, MPH, Division of General Pediatrics, University of Washington, Box 354920, 6200 NE 74th St., Suite 110, Seattle, WA 98118 (ddenno@uw.edu).

Journal of the Pediatric Infectious Diseases Society 2017;6(3):e49–e54

© The Author 2017. Published by the Oxford University Press on behalf of The Journal of the Pediatric Infectious Diseases Society. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/jpids/piw083

MATERIALS AND METHODS

Study Subjects and Baseline Pathogen Testing

We used materials and data from a subset of subjects from our 2003–2005 prospective study, in which children who presented to SCH with diarrhea were matched to healthy community-based controls according to age, sex, and geography, as described in detail in a previous report [6]. We failed to assign a definite or plausible etiology to nearly one-third of the subjects with diarrhea in the original study, in which viral antigen

detection, microscopy (parasites), and bacterial culture and toxin testing were used [6]. In this new study, we analyzed stool samples, on the basis of availability of sufficient specimen, from 178 of those cases and 178 matched controls (randomly selected if specimens from more than 1 control were available). These 356 subjects are termed the core group.

Testing of stool samples from the core group consisted of PCR for classic, VA, and MLB astrovirus clades (pan-astrovirus PCR), noroviruses GI and GII, and HBoVs 2, 3, and 4. We also tested stool samples from a subset of 98 randomly selected cases and their 98 matched controls for MW and STL PyVs by PCR (resource limitations precluded testing the entire core group for these organisms).

The stool samples had been frozen since acquisition. When an aliquot from a vial was removed for analysis, a small piece from the frozen primary sample was chipped without thawing. To detect viruses, we removed 80 mg of frozen stool and extracted RNA and DNA from the 200-μL fluid portion of diluted and lysed samples (NucliSens easy-MAG extractor [bioMérieux, Durham, NC]). Quantitative reverse-transcriptase/quantitative PCR was performed using an Applied Biosystems (Foster City, CA) 7300 real-time PCR system thermocycler for noroviruses GI and GII and HBoVs 2, 3, and 4 and the Applied Biosystems 7300 and 7500 systems for MW and STL PyVs. Amplifications that exceeded the cycle threshold were considered positive. We searched for astroviruses using primers that target virus RNA-dependent RNA polymerase using conventional reverse-transcription PCR [7]; amplicons were cloned and sequenced to identify the astrovirus clade (see Supplementary Table S1).

Assignment to Bona fide or Candidate Pathogen Status

Bona fide pathogens were defined as a bacterium or virus that, on the basis of recent literature [6, 8], is generally considered to cause diarrhea when detected during an acute diarrheal illness. Specifically, *Campylobacter* spp, O157:H7 and non-O157:H7 Shiga toxin-producing *E coli*, *Salmonella* spp, *Shigella* spp, *Yersinia* spp, adenovirus 40/41, classic astrovirus, noroviruses GI and GII, and rotavirus were counted as bona fide pathogens. Candidate pathogens were defined as a bacterium or virus that can cause diarrhea but might also be found frequently in an asymptomatic person's stools (ie, *Clostridium difficile*, enteroaggregative *E coli*, MW and STL PyVs, and HBoVs 2, 3, and 4). Parasites were not identified often in our previous study [1] and, contrary to our expectations, were associated more frequently with control status (particularly *Cryptosporidium*). For this reason, we considered parasites in the aggregate as candidate, and not bona fide, pathogens.

Statistical Analysis

We used Stata 12 (StataCorp, College Station, TX) to test associations between presence of organisms and case status. Conditional logistic regression was used to determine univariate matched odds ratios (ORs) and adjusted ORs (aORs) and 95% confidence intervals (CIs). To address confounding by demographic and socioeconomic factors (including potential residual confounding by matching variables), we determined aORs and 95% CIs using multivariable conditional logistic models. However, point estimates and CIs were sometimes unstable when cells contained only 1 subject; in these instances, we calculated P values using the exact version of the McNemar test (P_{exact}).

RESULTS

Subjects

Demographic characteristics of the subjects (Table 1) resembled those of our larger previous study [6], from which they were randomly selected. Also, the subset group resembled the core group, from which it was also randomly selected. Controls were statistically more likely to be white, as in our previous work [6]. Ethnicity differed overall but not statistically so within matched sets. Household incomes according to zip code of residence did not differ significantly. However, because differences for both income and ethnicity went in the same direction as in the previous study, in which these differences were significant [6], we chose the conservative strategy of adjusting for race/ethnicity and median income according to zip code in the multivariable models. A median of 42 days (interquartile range, 10–121 days) elapsed between receipt of case stool samples and their corresponding control stool samples.

PCR Versus EIA

In the core group of 178 cases and 178 controls, PCR detected norovirus GII in the stool samples of 33 subjects in which this agent was not detected by an enzyme immunoassay (EIA) (Supplementary Table S2). In contrast, 9 stools that tested positive for norovirus GI or GII by EIA were not corroborated by PCR. Pan-astrovirus PCR results were positive for 3 subjects who were not detected by an astrovirus EIA, but PCR did not confirm the positive EIA results for stools from 6 subjects. We based the remainder of our analysis for norovirus and astrovirus on PCR results only.

Viral Pathogen Prevalence

Norovirus serotypes GI and GII were found more often among cases (Table 2); the association with case status was statistically significant for both serotypes combined and for GII individually. HBoVs 2, 3, and 4 were recovered more often in case than in control specimens but were also the most commonly identified organism of interest in control stools; the difference between cases and controls was not statistically significant. Astroviruses (6 classic and 1 VA2) and adenovirus were identified more frequently in case stools than in control stools, but these differences also were not statistically significant. Rotavirus

Table 1. Demographic Characteristics for the Core and Subset Groups

	Core Group		Subset Group	
Characteristic	Cases	Controls	Cases	Controls
N	178	178	98	98
Female sex (n [%])	90 (51)	86[a] (49)	48 (49)	47[a] (48)
Age (mo)	44.8 (11–60)	43.8 (11–59)	48.2 (12–67)	47.6 (11–65)
Race (n [%])[b]				
White	95 (53)	130 (73)	51 (52)	72 (73)
African American	22 (12)	17 (10)	13 (13)	10 (10)
Asian/Pacific Islander	21 (12)	24 (13)	14 (14)	14 (14)
Native American	4 (2)	2 (1)	2 (2)	2 (2)
Other	33 (19)	19 (11)	13 (13)	10 (10)
Don't know	17 (10)	1 (0.6)	12 (12)	0 (0)
No response	17 (10)	2 (1)	12 (12)	0 (0)
Ethnicity (n [%])				
Hispanic	47 (26)	34 (19)	23 (24)	19 (19)
Don't know	8 (4)	1 (0.6)	6 (6)	0 (0)
No response	0 (0)	1 (0.6)	0 (0)	0 (0)
Demographics				
Household income (thousands of US dollars)[c]	48.8 (43–62)	56 (43–64)	50.2 (45–64)	56.7 (46–64)
Case and control proximity[d]	56 (31%), 66 (37%), 24 (13%)		32 (33%), 38 (39%), 12 (12%)	

[a]Sex was not provided for 1 control subject.
[b]Percentages exceed 100 because of multiple responses by some subjects.
[c]Based on 1999 US census median income of households in the participants' zip code of residence.
[d]Case and control matched sets residing in same, contiguous, or contiguous to contiguous zip codes.

was strongly associated with case status. In the subset group, MW PyV was as common among control stools as among case stools, and STL PyV was found in only 1 subject (a control).

Overall, 141 (79.2%) of the core-group case stool samples and 43 (24.2%) of the control stool samples tested positive for at least 1 bona fide or candidate pathogen (excluding the PyVs, which were not sought among all core-group stool samples). Multiple organisms were recovered in 35 (20%) of the case and 3 (2%) of the control stool samples (Supplementary Table S3).

Nearly half of the norovirus-positive specimens and more than three-quarters of the HBoVs 2, 3, and 4–positive case specimens were positive for an additional organism of interest, compared to only one-third of rotavirus-containing or bona fide bacterial pathogen-containing case stools. Nearly half of the coidentified agents in the rotavirus- or bona fide bacterial pathogen-containing stools were bona fide pathogens, whereas nearly three-quarters and all the coidentified agents in the norovirus-containing and HBoVs 2, 3, and 4–containing stools, respectively, were bona fide pathogens.

Supplementary Table S4 describes the characteristics of illnesses in which noroviruses GI and GII and HBoVs 2, 3, and 4 were identified and illnesses in which neither a bona fide nor candidate pathogen was detected. No pattern of illness characteristics that was unique to any given candidate viral pathogen emerged. Antibiotic use by cases in the 30 days before the onset of diarrhea was reported at rates similar to or higher than those among controls in the 30 days preceding study enrollment across every type of organism studied. Across the entire study, reported case antibiotic use was more than 4-fold higher than that among controls (adjusted OR, 4.5 [95% CI, 2.3–8.5]) (Supplementary Table S5).

DISCUSSION

Viral PCR data from this study update our previous findings on causes of childhood acute diarrhea. We reduced the frequency of undetected bona fide or candidate fecal pathogens among cases from 32% to 21%. In the process, we found that noroviruses are second only to rotavirus as causes of diarrhea in a pediatric emergency department in Seattle, just before introduction of the currently used rotavirus vaccines. Specifically, the percentages of case (18.8%) and control (4.5%) specimens that tested positive for norovirus resemble the rates reported from a recent surveillance study from 3 counties in the United States [9], which was conducted after implementation of the rotavirus vaccine. Our GII carriage rate among healthy controls is similar also to that of children in that multicenter study (9). These data suggest that rotavirus vaccination was not associated with a profound increase in the proportion of diarrhea episodes in children that can be attributed to norovirus.

We detected a VA2 clade astrovirus in a single case stool. VA2 is 1 of 8 divergent enteric astroviruses that have been found in stools since their discovery in 2008 [3, 7, 10–15]. Broadly reactive primers, such as those used here, could be useful in efforts

Table 2. Organisms of Interest Found in Core (178 Cases, 178 Controls) and Subset (98 Cases, 98 Controls) Group Stool Specimens

Agent (Detection Methodology)	No. (%) of Positive Results		Unadjusted OR (95% CI) or *P*	Adjusted[a] OR (95% CI)
	Cases	Controls		
Viruses (core group)				
Norovirus GI (PCR)	4 (2.2)	0 (0)	0.13[b]	NA
Norovirus GII (PCR)	30 (16.9)	8 (4.5)	4.7 (1.9–11.3)	4.7 (2.2–10.1)
Norovirus GI and/or GII (PCR)	33 (18.5)	8 (4.5)	5.2 (2.2–12.4)	5.3 (2.5–11.3)
HBoVs 2, 3, and 4 (PCR)	22 (12.4)	14 (7.9)	1.6 (0.8–3.2)	1.7 (0.8–3.6)
Pan-astrovirus (PCR)	6 (3.3)[c]	1 (0.5)	6.0 (0.7–50.1)	6.8 (0.9–51.2)
Adenovirus (EIA)	7 (3.9)	2 (1.1)	3.5 (0.7–16.9)	3.5 (0.8–16.1)
Rotavirus (EIA)	73 (41.0)	1 (0.6)	<0.001[b]	NA
MW and STL PyV (subset group)				
MW PyV (PCR)	10 (10.2)	10 (10.2)	1 (0.4–2.5)	1.1 (0.4–3.0)
STL PyV (PCR)	0 (0)	1 (1.0)	1.0[b]	NA
Bacterial pathogens (core group)				
Any bacterial pathogen	21 (11.8)	NA[d]	NA[d]	NA[d]
Campylobacter (culture)	4 (2.2)[e]	NA[d]	NA[d]	NA[d]
Salmonella (culture)	8 (4.5)[f]	NA[d]	NA[d]	NA[d]
Shigella (culture)	2 (1.1)[g]	NA[d]	NA[d]	NA[d]
E coli O157:H7[h] (culture)	5 (2.8)	NA[d]	NA[d]	NA[d]
Non-O157:H7[i] STEC (EIA and culture)	3 (1.7)[j,k]	NA[d]	NA[d]	NA[d]
STEC (EIA[l])	7 (3.9)	0 (0)	0.02[c]	NA
EAggEC (PCR)	7 (3.9)	2 (1.1)	3.5 (0.7–16.9)	4.3 (1.0–18.8)
C difficile (cytotoxicity)	11 (6.2)	13 (7.3)	0.8 (0.3–2.0)	0.7 (0.3–1.8)
Parasites (core group)				
Any parasite (microscopy)[m]	1 (0.6)	7 (4.0)	0.1 (0.0–1.2)	0.2 (0.0–1.3)

Abbreviations: CI, confidence interval; EAggEC, enteroaggregative *E coli*; EIA, enzyme immunoassay; HBoV, human bocavirus; MW, Malawi; OR, odds ratio; PCR, polymerase chain reaction; PyV, polyomavirus; STEC, Shiga toxin-producing *E coli*; STL, St Louis.

[a]Adjusted for mean income according to zip code and race/ethnicity.

[b]For organisms for which ≤ 1 subject's specimen tested positive and OR estimates were unstable, we present *P* values from an exact version of the McNemar test rather than ORs and CIs.

[c]All astroviruses were classic, except for 1 case stool that contained Virginia 2 clade and *Campylobacter*. Restricting the analysis to only the classic astroviruses did not alter the calculated OR, adjusted OR, or 95% CIs meaningfully.

[d]Not applicable because controls were not tested for this agent.

[e]Three of these cases were infected with *Campylobacter jejuni*.

[f]Includes *Salmonella enterica* subsp *enterica* serovar Typhimurium (3), *S enterica* subsp *enterica* serovar Heidelberg (2), *S enterica* subsp *enterica* serovar Brandenburg, *S enterica* subsp *enterica* serovar Newport, and *Salmonella* serotype I 4,5,12:i:- (1 each).

[g]Both *Shigella sonnei*.

[h]As identified on sorbitol MacConkey agar; the broth culture of 1 subject tested negative for Stx (EIA).

[i]Isolates from broth cultures that tested positive for Stx (EIA).

[j]One case-subject was infected with STEC O111:nonmotile and *S enterica* subsp *enterica* serovar Brandenburg.

[k]*E coli* isolates belonged to serotypes O111:nonmotile, O121:nonmotile, and O177:nonmotile (1 each).

[l]Defined as subjects whose broth cultures tested positive for Shiga toxin (Stx) (EIA) and from which a non-O157:H7 or an O157:H7 STEC was recovered but does not include the additional case subject whose stool contained STEC O157:H7, as identified on sorbitol MacConkey agar, but whose toxin assay result was negative.

[m]*Cryptosporidia* spp (1 case and 4 control subjects) and *Entamoeba coli*, *Blastocystis hominis*, and *Endolimax nana* (1 control subject each).

to further explore the role of emerging astroviruses in human disease. Our data also do not support MW and STL PyVs as causes of childhood diarrhea.

The overrepresentation of HBoVs 2, 3, and 4 in case stools warrants comment. HBoVs were first reported as pathogens in 2005 [16], when an agent now termed HBoV serotype 1 was recovered from respiratory secretions. HBoV serotype 2 and, to lesser extents, serotypes 1, 3, and 4 were subsequently identified in human stool [2, 17]. Although HBoV 2 was associated with childhood gastroenteritis in Australia [17], additional studies found a weaker or no association between fecal excretion of HBoVs and gastroenteritis [1, 18–22]. It is difficult to draw firm conclusions from these studies, because HBoV was often present in combination with other pathogens, control groups of convenience were used, and primer choices were variable. Although our analysis identified HBoVs 2, 3, and 4 more frequently among cases, this association did not reach statistical significance, perhaps because the power was inadequate.

We found multiple bona fide and/or candidate pathogens in many specimens, as have others [23–27], particularly for HBoVs 2, 3, and 4, for which more than 80% of stools that contained this agent also contained another organism of interest, usually a virus. As we noted already, such findings cast doubt on the causative role of these agents. It is interesting to note, however, that it is common to find coinfecting agents when HBoV 1 is identified in the airway [28], which suggests that helper viruses play roles in HBoV biology or that HBoV is, itself, a helper virus for other agents. More studies of HBoVs 2, 3, and

4, including refinement of PCR-based diagnostics for these viruses, are needed before concluding that HBoVs 2, 3, and 4 do not cause childhood gastroenteritis [29]. Also, further work to determine the role of multiple agents in illness is warranted.

Despite expanding our test repertoire to include PCR for multiple viruses, many cases of diarrhea remain undiagnosed. Nonetheless, our data reduced the percentage of cases without a plausible or definite pathogen to 21% from 32% in the predecessor study. Continued pathogen discovery is necessary to identify the cause of this one-fifth of cases, although some of these episodes might not have been infectious in origin. Clarification of microbial and host factors that influence clinical disease among the colonized is also needed.

It is interesting to note that case-subjects had a 4.5-fold greater odds of antibiotic exposure in the 30 days before the onset of diarrhea than did the controls in the 30 days before study entry. Along these lines, antibiotic use in young Indian children was found to diminish time to the next diarrhea episode [30]. The reasons for such effects are unclear but might be attributable to intestinal dysbiosis.

Our data have relevance for the approaching era of nonculture and panel diagnostics. These technologies present opportunities for enteric infection detection and management, but they also present challenges, chiefly in the domain of the clinical relevance of positive results. Nucleic acid tests of stool are quite sensitive and will generate more positive results than those of traditional technologies; the relationship between some of these positive results and patient illness will be difficult to discern. Clinicians will be confronted with test results that might be positive as a result of nucleic acid amplification from a nonviable pathogen. Many organisms of interest, including bona fide pathogens, are excreted at appreciable rates by children without diarrhea. These factors should be taken into account before attributing an episode of diarrhea to any detected agent. It will be important to determine if pathogen burden (ie, multiple different agents and concentration of infecting organisms of interest per gram of stool) relates to causality. Quantitative PCR testing might offer a particular opportunity in this regard, because a low cycle threshold, consistent with a high level of virus, might add increased specificity when assigning clinical relevance. Also, although the reliance of our specimen selection for each group depended on specimen availability, such a bias exists in many studies of childhood diarrhea etiology. For example, Klein et al [8] reported that in a study of children who presented to a pediatric emergency department with diarrhea, only 372 (22.9%) of 1626 specimens submitted were of sufficient quantity to conduct studies that required bulk stool analysis for a panel of different agents. Future studies of childhood diarrhea should attempt to address the equivalency of molecular analyses on swab specimens to assays performed on cup specimens.

In summary, the results of our work support the importance of norovirus as a cause of community-acquired childhood diarrhea. The more frequent detection of HBoVs 2, 3, and 4 in children with diarrhea compared to that in controls warrants serotype-specific investigation. Nonclassic astroviruses and STL PyV were uncommon, and MW PyV was not associated with childhood diarrhea. We need more data to better interpret the presence of multiple agents in stools of children with diarrhea, because most knowledge about childhood diarrhea is based on single-agent data. Future studies of childhood diarrhea should be designed to address the potential synergistic role of coinfections. Last, 24% of our control-subjects shed an organism of interest in their stools; the role of asymptomatic shedding of such agents in disease transmission in the community merits further investigation.

Supplementary Data

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

Notes

Acknowledgments. We thank the subjects and their families; the SCH emergency department nurses and physicians and the SCH microbiology laboratory staff for their assistance with this complex project; Nurmohammad Shaikh and Carla Hall-Moore for materials management; Maria Cannella, Richard Buller, Cynthia Rodriguez, Irma Bauer, Jenny Stapp, Xuan Qin, Valerie Hoffman, Jody Mooney, and Kelly Wood for specimen handling and technical assistance; and Maida Redzic for assistance with manuscript preparation. We particularly thank the network of community-based controls, their families, and their primary care doctor offices and staff for their enthusiastic assistance with the recruitment of this unique control specimen set.

Financial support. This work was supported by the US Department of Agriculture National Research Initiative (grant 0202238 to P. I. T.), the Biobank Core of the Washington University School of Medicine Digestive Diseases Research Core Center (grant P30DK052574), and the Children's Discovery Institute (grant MD-FR-2013–292 to L. R. H.). Virus enzyme immunoassay kits were kindly provided at reduced cost by Dako, but the company had no role in study design or manuscript preparation.

Potential conflicts of interest. All authors: No reported conflicts

All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Chow BD, Ou Z, Esper FP. Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the United States. J Clin Virol **2010**; 47:143–7.
2. Kapoor A, Simmonds P, Slikas E, et al. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis **2010**; 201:1633–43.
3. Meyer CT, Bauer IK, Antonio M, et al. Prevalence of classic, MLB-clade and VA-clade astroviruses in Kenya and The Gambia. Virol J **2015**; 12:78.
4. Siebrasse EA, Reyes A, Lim ES, et al. Identification of MW polyomavirus, a novel polyomavirus in human stool. J Virol **2012**; 86:10321–6.
5. Lim ES, Reyes A, Antonio M, et al. Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing. Virology **2013**; 436:295–303.
6. Denno DM, Shaikh N, Stapp JR, et al. Diarrhea etiology in a pediatric emergency department: a case control study. Clin Infect Dis **2012**; 55:897–904.
7. Finkbeiner SR, Le BM, Holtz LR, et al. Detection of newly described astrovirus MLB1 in stool samples from children. Emerg Infect Dis **2009**; 15:441–4.
8. Klein EJ, Boster DR, Stapp JR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. Clin Infect Dis **2006**; 43:807–13.
9. Payne DC, Vinje J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. N Engl J Med **2013**; 368:1121–30.
10. Phan TG, Nordgren J, Ouermi D, et al. New astrovirus in human feces from Burkina Faso. J Clin Virol **2014**; 60:161–4.

11. Jiang H, Holtz LR, Bauer I, et al. Comparison of novel MLB-clade, VA-clade and classic human astroviruses highlights constrained evolution of the classic human astrovirus nonstructural genes. Virology **2013**; 436:8–14.
12. Finkbeiner SR, Holtz LR, Jiang Y, et al. Human stool contains a previously unrecognized diversity of novel astroviruses. Virol J **2009**; 6:161.
13. Finkbeiner SR, Li Y, Ruone S, et al. Identification of a novel astrovirus (astrovirus VA1) associated with an outbreak of acute gastroenteritis. J Virol **2009**; 83:10836–9.
14. Kapoor A, Li L, Victoria J, et al. Multiple novel astrovirus species in human stool. J Gen Virol **2009**; 90:2965–72.
15. Finkbeiner SR, Kirkwood CD, Wang D. Complete genome sequence of a highly divergent astrovirus isolated from a child with acute diarrhea. Virol J **2008**; 5:117.
16. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A **2005**; 102:12891–6.
17. Arthur JL, Higgins GD, Davidson GP, et al. A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog **2009**; 5:e1000391.
18. Cheng WX, Jin Y, Duan ZJ, et al. Human bocavirus in children hospitalized for acute gastroenteritis: a case-control study. Clin Infect Dis **2008**; 47:161–7.
19. Jin Y, Cheng WX, Xu ZQ, et al. High prevalence of human bocavirus 2 and its role in childhood acute gastroenteritis in China. J Clin Virol **2011**; 52:251–3.
20. La Rosa G, Della Libera S, Iaconelli M, et al. Human bocavirus in children with acutegastroenteritis in Albania. J Med Virol **2015**; 88:906–10.
21. Campos GS, Silva Sampaio ML, Menezes AD, et al. Human bocavirus in acute gastroenteritis in children in Brazil. J Med Virol **2016**; 88:166–70.
22. Paloniemi M, Lappalainen S, Salminen M, et al. Human bocaviruses are commonly found in stools of hospitalized children without causal association to acute gastroenteritis. Eur J Pediatr **2014**; 173:1051–7.
23. de Boer RF, Ott A, Kesztyus B, Kooistra-Smid AM. Improved detection of five major gastrointestinal pathogens by use of a molecular screening approach. J Clin Microbiol **2010**; 48:4140–6.
24. Ardissino G, Possenti I, Salardi S, et al. Co-infection in children with bloody diarrhea caused by Shiga toxin-producing *Escherichia coli*: data of the North Italian HUS Network. J Pediatr Gastroenterol Nutr **2014**; 59:218–20.
25. Garg AX, Macnab J, Clark W, et al. Long-term health sequelae following *E. coli* and *Campylobacter* contamination of municipal water. Population sampling and assessing non-participation biases. Can J Public Health **2005**; 96:125–30.
26. Luna-Gierke RE, Wymore K, Sadlowski J, et al. Multiple-aetiology enteric infections involving non-O157 Shiga toxin-producing *Escherichia coli*—FoodNet, 2001–2010. Zoonoses Public Health **2014**; 61:492–8.
27. Bopp DJ, Sauders BD, Waring AL, et al. Detection, isolation, and molecular subtyping of *Escherichia coli* O157:H7 and *Campylobacter jejuni* associated with a large waterborne outbreak. Eur J Clin Microbiol **2003**; 41:174–80.
28. Jartti T, Hedman K, Jartti L, et al. Human bocavirus—the first 5 years. Rev Med Virol **2012**; 22:46–64.
29. Padhye A, Storch GA. Relationship of human bocavirus 2 to gastroenteritis in children. J Clin Virol **2016**; 80:72–3.
30. Rogawski ET, Westreich DJ, Becker-Dreps S, et al. Antibiotic treatment of diarrhoea is associated with decreased time to the next diarrhoea episode among young children in Vellore, India. Int J Epidemiol **2015**; 44:978–87.