MAJOR ARTICLE

Etiology of Viral Gastroenteritis in Children <5 Years of Age in the United States, 2008–2009

Preeti Chhabra,[1] Daniel C. Payne,[2] Peter G. Szilagyi,[4] Kathryn M. Edwards,[5] Mary Allen Staat,[6] S. Hannah Shirley,[1] Mary Wikswo,[2] W. Allan Nix,[3] Xiaoyan Lu,[1] Umesh D. Parashar,[2] and Jan Vinjé[1]

[1]Gastroenteritis and Respiratory Viruses Laboratory Branch, [2]Epidemiology Branch, and [3]Polio and Picornavirus Laboratory Branch, Division of Viral Diseases, National Center for Immunizations and Respiratory Disease, Centers for Disease Control and Prevention, Atlanta, Georgia; [4]Department of Pediatrics, University of Rochester School of Medicine and Dentistry, New York; [5]Department of Pediatrics, Vanderbilt Vaccine Research Program, Vanderbilt University Medical Center, Nashville, Tennessee; and [6]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati Children's Hospital Medical Center, Ohio

Background. Although rotavirus and norovirus cause nearly 40% of severe endemic acute gastroenteritis (AGE) in children <5 years of age in the United States, there are limited data on the etiologic role of other enteric viruses in this age group.

Methods. We conducted active population-based surveillance in children presenting with AGE to hospitals, emergency departments, and primary care clinics in 3 US counties. Stool specimens from these children and from age-matched healthy controls collected between October 2008 and September 2009 were tested for enteric adenovirus, astrovirus, sapovirus, parechovirus, bocavirus, and aichivirus. Typing was performed by sequencing and phylogenetic analysis.

Results. Adenovirus, astrovirus, sapovirus, parechovirus, bocavirus, and aichivirus were detected in the stool specimens of 11.8%, 4.9%, 5.4%, 4.8%, 1.4%, and 0.2% of patients with AGE and 1.8%, 3.0%, 4.2%, 4.4%, 2.4%, and 0% of healthy controls, respectively. Adenovirus (type 41), astrovirus (types 1, 2, 3, 4, and 8), sapovirus (genogroups I and II), parechovirus (types 1, 3, 4, and 5), and bocavirus (types 1, 2, and 3) were found cocirculating.

Conclusions. Adenovirus, astrovirus, and sapovirus infections were detected in 22.1% of the specimens from children <5 years of age who had medical visits for AGE and tested negative for rotavirus and norovirus. No causal role for parechovirus and bocavirus was found.

Keywords. viral gastroenteritis; sapovirus; astrovirus; adenovirus; population-based surveillance.

Acute gastroenteritis (AGE) is a major cause of morbidity and mortality in pediatric populations worldwide. Globally, an estimated 800 000 infants and young children die from diarrhea each year [1]. Mortality is uncommon in developed countries, but diarrhea is often associated with substantial medical and healthcare costs and thus has a high economic impact on society [2]. According to recent estimates, nearly 179 million gastrointestinal illnesses occur each year in the United States, of which 141.8 million (approximately 80%) are caused by unspecified and/or unknown agents [3].

Viruses are the major etiological agents of AGE in children <5 years of age. Group A rotavirus, norovirus, enteric adenovirus, human astrovirus, and sapovirus are established etiological agents of AGE. Other viruses, such as aichivirus, human parechovirus, and human bocavirus, have recently been described in patients with diarrhea [4–8], but their association with AGE has not yet been established as most data have been reported in symptomatic individuals only and did not include age-matched healthy controls. It is important to identify specific etiologies of AGE in order to target potential preventive interventions, such as vaccinations.

In the United States, most data on the viral etiology of AGE in children <5 years of age are available for

Received 15 January 2013; accepted 27 February 2013; electronically published 10 June 2013.

Correspondence: Jan Vinjé, PhD, Division of Viral Diseases, Centers for Disease Control and Prevention, 160 Clifton Rd, Atlanta, GA 30333 (jvinje@cdc.gov).

The Journal of Infectious Diseases 2013;208:790–800

DOI: 10.1093/infdis/jit254

rotavirus and norovirus [9–13], with very few reports on the role of adenovirus, astrovirus, and sapovirus [14–17].

To assess the impact of the US rotavirus vaccination program, population-based active surveillance for AGE has been conducted in young children in 3 US counties since 2006. In fecal specimens from symptomatic children with AGE collected between October 2008 and September 2009, we recently reported the well-recognized enteric viral pathogens, rotavirus and norovirus, in a total of 39.4% of samples from children with AGE [12]. This surveillance program also provides a unique opportunity to examine the role that other pathogens and novel viruses with unknown pathogenicity play in children with AGE. In the current study, we expanded testing for additional enteric viruses to include adenovirus, astrovirus, sapovirus, bocavirus, parechovirus, and aichivirus and compared their prevalence among age-matched healthy controls to determine whether these viruses were associated with clinical illness.

METHODS

NVSN Surveillance Network

The New Vaccine Surveillance Network (NVSN) conducts active, population-based surveillance on viral causes of AGE in healthy children <5 years of age seen in hospitals, emergency departments (EDs), and selected outpatient clinics in 3 US counties, as previously described [10, 11]. The 3 sites—University of Rochester (Rochester, NY), Vanderbilt University (Nashville, TN), and Cincinnati Children's Hospital Medical Center (Cincinnati, OH)—together cover a catchment population of about 141 000 children <5 years of age.

Children between 15 days and 5 years of age with symptoms of AGE (i.e., diarrhea [3 episodes within 24 hours] and/or vomiting [any episodes within 24 hours] lasting for ≤10 days) who visited hospitals, EDs, and outpatient clinics at the 3 sites from October 2008 through September 2009 were eligible to be enrolled in the study. Stool specimens were obtained within 14 days of the date of visitation or admission for AGE symptoms (95% specimens were collected within 7 days).

Healthy controls were enrolled at the same sites during routine well-child visits in the same period. Healthy controls were <5 years old, had no parental report of existing chronic conditions, and had no diarrhea or vomiting within 14 days of enrollment. Stool specimens from healthy controls were frequency matched to those of patients with AGE on the basis of age in months and were collected within 5 days of enrollment. Informed consent was obtained from the parents or guardians of enrolled children. Institutional review board approvals were obtained from the Centers for Disease Control and Prevention and from each study site.

Clinical and epidemiological data about the onset and duration of diarrhea, vomiting, fever, and treatment (oral rehydration/intravenous fluids) were analyzed to calculate the severity score by a modified 20-point scale of the Vesikari system [18]. According to the scores obtained, the disease condition of each patient was categorized as mild (score, 0–10), moderate (score, 11–14), or severe (score, 15–20).

Laboratory Testing

All specimens had been tested previously for group A rotavirus by an enzyme immunoassay kit (Premier Rotaclone, Meridian Bioscience, Cincinnati, OH) and for norovirus by a real-time TaqMan reverse transcription polymerase chain reaction (RT-PCR) assay [12, 19]. We tested 782 samples from children with AGE and 499 samples from healthy controls.

Viral nucleic acid was extracted from 10% clarified stool suspensions prepared in phosphate-buffered saline, using the MagMax-96 Viral RNA Isolation Kit (Ambion, Foster City, CA) according to the manufacturer's instructions. All extractions were performed on an automated KingFisher magnetic particle processor (Thermo Fisher Scientific, Pittsburgh, PA). The presence of adenovirus, astrovirus, sapovirus, human parechovirus, human bocavirus, and aichivirus was determined by real-time RT-PCR or PCR, using the AgPath-ID One-Step RT-PCR kit (Applied Biosystems, Foster City, CA) and virus-specific oligonucleotide primers and probes (Table 1). For RNA viruses, reverse transcription was performed at 45°C for 10 minutes, followed by inactivation of reverse transcriptase at 95°C for 10 minutes. PCR cycling conditions for all viruses included 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute.

Typing of positive samples was performed by conventional PCR or RT-PCR, using the OneStep RT-PCR kit (Qiagen, Valencia, CA) and virus-specific oligonucleotide primers (Table 1). RT-PCR products were separated on 2% agarose gels, and the amplicons of appropriate sizes were purified using the QIAquick gel extraction kit (Qiagen), followed by cycle sequencing with BigDye Terminator v1.1 and 3130XL automated DNA sequencer (Applied Biosystems). Multiple sequence alignments were performed using ClustalW [29], and phylogenetic analysis was done using MEGA 4 [30]. Phylogenetic trees were generated using the neighbor-joining algorithm and Kimura 2-parameter distance model. The reliability of the phylogenetic trees was tested by applying 1000 bootstrap replicates.

Statistical Analyses

We determined the prevalence of each virus in AGE samples and determined whether there were statistically significant differences between the proportion of patients with AGE and healthy controls with virus detected, using a χ^2 test and a Fisher exact test; P values of $< .05$ were considered statistically significant.

RESULTS

Prevalence of Specific Viruses

Overall, 782 children with AGE (324 who were hospitalized, 342 who presented to the ED, 116 who visited an outpatient

Table 1. Oligonucleotide Primers and Probes Used for Detection and Genotyping of Enteric Viruses by Real-Time and Conventional Reverse Transcription Polymerase Chain Reaction

Target Virus, Target Region, Primer/Probe	Oligonucleotide Primer/Probe Sequence (5′-3′)	Amplicon Size (bp)	Reference
Sapovirus			
RdRp/Capsid (RT-qPCR)			
SaV124F	GAY CAS GCT CTC GCY ACC TAC	NA	[20]
SaV1F	TTG GCC CTC GCC ACC TAC		
SaV5F	TTT GAA CAA GCT GTG GCA TGC TAC		
SaV1245R	CCC TCC ATY TCA AAC ACT A		
SaV124TP	FAM-CCR CCT ATR AAC CA-[MGB-NQF]		
SaV5TP	FAM-TGC CAC CAA TGT ACC A-[MGB-NQF]		
VP1 (Conventional RT-PCR)			
SV-F13	GAY YWG GCY CTC GCY ACC TAC	802	[21]
SV-F14	GAA CAA GCT GTG GCA TGC TAC		
SV-R13	GGT GAN AYN CCA TTK TCC AT		
SV-R14	GGT GAG MMY CCA TTC TCC AT		
SV-F22	SMW AWT AGT GTT TGA RAT G	450	
SV-R2	GWG GGR TCA ACM CCW GGT GG		
Astrovirus			
RdRp (RT-qPCR)	GGC CAG ACT CAC AGA AGA GCA	NA	[22]
AsFF	GTC CTG TGA CAC CTT GTT TCC TGA		
AsFr	HEX-CCA TCG CAT TTG GAG GGG AGG ACC AGC GA-BHQ1		
AstZFb			
Capsid (Conventional RT-PCR)			
Mon 269	CAA CTC AGG AAA CAG GGT GT	449	[23]
Mon 270	TCA GAT GCA TTG TCA TTG GT		
Adenovirus			
Fiber (qPCR)			
JTVFF	AAC TTT CTC TCT TAA TAG ACG CC	NA	[24]
JTVFR	AGG GGG CTA GAA AAC AAA A		
JTVFAP	FAM-CGA AGA GTG CCC GTG TCA GC-BHQ1		
Hexon (Conventional PCR)			
Ad40F	GCC GCA GTG GTC TTA CAT GCA CAT C	300	[25]
Ad41R	CAG CAC GCC GCG GAT GTC AAA GT		
Aichivirus			
5′ UTR (RT-qPCR)			
AN490	TGG SGT AAG GTT SRT GTG CC	NA	This study
AN491	ATA TCT GAG AMG RYG TTC CGC TG		This study
AN492	ACA ATT AGC CCA GGY TCA GAT CC		This study
AN493P	FAM-GGY GGG GTA CCT SCC TGG CAT TCC TRG G-BHQ1		This study
VP1(Conventional RT-PCR)			
AN568	TNC CCC A	NA	This study
AN569	CKN GGA AC		This study
AN570	CKN GGT AC		This study
AN571	CKN GGG AC		This study
AN572	CKN GGC AC		This study
2A			
AN573	CNC CTT G		This study
AN574	CNC CCT G		This study
VP3			
AN560	CAR GCI TAY TGG AAR CAN GTN GA	587	This study
VP1			
AN561	GGI ACI CTI GCN CCN GGN GGN GC		This study

Table 1 continued.

Target Virus, Target Region, Primer/Probe	Oligonucleotide Primer/Probe Sequence (5′-3′)	Amplicon Size (bp)	Reference
VP3			
AN563	CTG CTT CGC AGG CCT ACT GGA ARA CNG TNG A	599	This study
VP1			
AN562	AGA AGG GAC AGA TGC ACC NGG NGG NGC		This study
Parechovirus			
5′ UTR (RT-qPCR)			
AN344	GGCCCCWGRTCAGATCCAYAGT	NA	[26]
AN345	GTAACASWWGCCTCTGGGSCCAAAAG		
AN257P	(HEX)-CCTRYGGGTACCTYCWGGGCATCCTTC-(BHQ1)		
2A (Conventional RT-PCR)			
AN273	AARTAGTC	NA	[27]
AN274	AARTAATC		
AN275	TCRCAGTT		
AN276	TCRCAATT		
2B			
AN277	ATRAATTT		
AN278	ATRAACTT		
VP1	GAATAAAATGGTACTGANARNGTCATYTGYTC	299	
AN357	ACC AAG GTT GAC AAC ATT TTY GGN MGN GC		
AN369			
Bocavirus			
5′-UTR/NS1 (qPCR)			
HBoV1F	CCT ATA TAA GCT GCT GCA CTT CCT G	NA	[28]
HBoV1R	AAG CCA TAG TAG ACT CAC CAC AAG		
HBoV234F	GCA CTT CCG CAT YTC GTC AG		
HBoV3R	GTG GAT TGA AAG CCA TAA TTT GA		
HBoV24R	AGC AGA AAA GGC CAT AGT GTC A		
HBoVP	FAM-5′-CCA GAG ATG TTC ACT CGC CG-BHQ1		
NS1 (Conventional PCR)			
HBoVF1	GAG CTT AAC CTA CTA GTD TAT GAA GAC TTA GT	459	This study
HBoVR1	CAT TAA GCA YTC YTC CCA CCA		This study
HBoVF2	GTA AAA CGA CGG CCA GTG TCC TGA ACT ACT CCT WAT GCT TGA A	419	This study
HBoVR2	CAG GAA ACA GCT ATG ACA GGC GTT GTC TGC ART CRT TAA A		This study

Abbreviations: BHQ1, black hole 1 quencher; FAM, 6-carboxyfluorescein; HEX, hexachloro fluorescein; MGB, minor groove binder; NA, not applicable; NQF, nonfluorescent quencher, RT-qPCR, reverse transcription-real-time polymerase chain reaction.

International Union of Biochemistry codes were used to denote mixed bases (e.g., Y = C or T). Sapovirus probes were labeled with FAM reporter dye at the 5′ end and with MGB-NQF at the 3′ end of the oligonucleotide. Aichivirus and bocavirus probes were labeled with FAM reporter dye at the 5′ end and with BHQ1 at the 3′ end of the oligonucleotide. Astrovirus and human parechovirus probes were labeled with HEX reporter dye at the 5′ end and with BHQ1 at the 3′ end of the oligonucleotide.

clinic) and 499 healthy controls were enrolled. At least 1 virus, including rotavirus (11.1%) and norovirus (14.5%) [12], was detected in 523 (40.8%) of 1281 children. Overall, adenovirus, astrovirus, and sapovirus were detected in 11.8%, 4.9%, and 5.4% of children with AGE, respectively, and in 1.8% ($P < .001$), 3% ($P < .001$), and 4.2% ($P < .01$) of the healthy controls, respectively (Table 2). Fifty-six (60%) of the 93 patients who tested positive for adenovirus were from the Rochester site. This site also showed higher detection rates for rotavirus (24.1%), norovirus (27.1%), and astrovirus (8.4%) in patients. Both parechovirus and bocavirus were infrequently found and had comparable prevalence (4.8% and 1.4%, respectively) in cases and healthy controls (4.4% [$P < .5$] and 2.4% [$P < .01$], respectively). Aichivirus was only detected in 2 children (0.2%) with AGE and was not detected in controls (Table 2).

Coinfection with >1 virus, including rotavirus and norovirus [12], was identified in 14.5% of all positive specimens, including 13.1% of specimens from patients with AGE and 1.3% from healthy controls. Both children with aichivirus detected in their stool and 70% of the parechovirus-positive specimens tested

Table 2. Rates of Positivity for Rotavirus, Norovirus, Sapovirus, Astrovirus, Adenovirus, Aichivirus, Parechovirus, and Bocavirus Among Children With Acute Gastroenteritis and Healthy Controls

	Cincinnati (n = 562)		Nashville (n = 376)		Rochester (n = 343)		Total (n = 1281)	
Virus	Case (n = 369)	Control (n = 193)	Case (n = 210)	Control (n = 166)	Case (n = 203)	Control (n = 140)	Case (n = 782)	Control (n = 499)
Rotavirus[a]	51 (13.8)	0	41 (19.5)	0	49 (24.1)	1 (0.7)	141 (18.0)	1 (0.2)
Norovirus[a]	71 (19.2)	8 (4.1)	41 (19.5)	3 (1.8)	55 (27.1)	9 (6.4)	167 (21.4)	20 (4.0)
Sapovirus	22 (6.0)	17 (8.8)	9 (4.3)	2 (1.2)	7 (3.4)	2 (1.4)	42 (5.4)	21 (4.2)
Astrovirus	16 (4.3)	3 (1.6)	4 (1.9)	3 (1.8)	17 (8.4)	9 (6.4)	38 (4.9)	15 (3.0)
Adenovirus	25 (6.7)	4 (2)	12 (5.7)	3 (1.8)	56 (27.5)	2 (1.4)	93 (11.8)	9 (1.8)
Aichivirus	2 (0.5)	0	0	0	0	0	2 (0.2)	0
Parechovirus	28 (7.5)	13 (6.7)	5 (2.3)	6 (3.6)	5 (2.4)	3 (2.1)	38 (4.8)	22 (4.4)
Bocavirus	8 (2.1)	6 (3.1)	0	5 (3)	3 (1.4)	1 (0.7)	11 (1.4)	12 (2.4)

Data are percentage of subjects.

[a] Data were previously published [11, 12] and are included for comparison.

positive for at least 1 other virus. Overall, norovirus was the most frequently detected pathogen (57.8%) in mixed infections (Table 3), with the combinations norovirus-astrovirus (15.7%) and norovirus-adenovirus (15.7%) most commonly seen.

Table 3. Mixed Infections Identified in 76 Fecal Specimens From Patients With Acute Gastroenteritis and Healthy Controls

Viruses[a]	Specimens, No.	Percentage Positivity
Rotavirus, norovirus	2	2.63
Rotavirus, sapovirus	4	5.26
Rotavirus, astrovirus	1	1.32
Rotavirus, adenovirus	2	2.63
Rotavirus, parechovirus	4	5.26
Rotavirus, bocavirus	3	3.95
Norovirus, sapovirus	5	6.58
Norovirus, astrovirus	12	15.79
Norovirus, adenovirus	12	15.79
Norovirus, aichivirus	1	1.32
Norovirus, parechovirus	9	11.84
Norovirus, bocavirus	1	1.32
Sapovirus, astrovirus	1	1.32
Sapovirus, adenovirus	3	3.95
Sapovirus, parechovirus	3	3.95
Sapovirus, bocavirus	1	1.32
Astrovirus, adenovirus	5	6.58
Parechovirus, bocavirus	1	1.32
Astrovirus, adenovirus, parechovirus	2	2.63
Norovirus, sapovirus, parechovirus	1	1.32
Norovirus, aichivirus, parechovirus	1	1.32
Sapovirus, adenovirus, parechovirus	1	1.32
Sapovirus, astrovirus, bocavirus	1	1.32

[a] Previously published rotavirus and norovirus data [11, 12] are included.

Clinical and Patient Characteristics Associated With Viral AGE

Seventeen (89.4%) of the 19 patients with sapovirus as a single pathogen reported vomiting, while 12 (63.1%) of the patients had diarrhea (Table 4). Ten (62.5%) of 16 patients with astrovirus infections were afebrile. Overall, the majority (89.3%) of the patients with adenovirus, astrovirus, or sapovirus infections as a single pathogen presented with mild (score, 0–10) to moderate (score, 11–14) disease severity, while 71.3% of the patients with rotavirus and norovirus infections presented with moderate (score, 11–14) to severe (score, 15–20) disease severity (Table 4).

The rate of adenovirus, astrovirus, and sapovirus detection decreased with increased age and was more frequent in children ≤2 years of age in patients (76.9%; median age, 14.6 months) and healthy controls (84.6%; median age, 11.5 months; Figure 1). None of these viruses were detected in healthy controls aged 4–5 years (Figure 1). The median age of sapovirus- and astrovirus-positive patients was 18 months as compared to 6 and 8 months, respectively, for sapovirus- and astrovirus-positive healthy controls ($P \leq .05$). However, no significant difference was found in the median age of patients and controls positive for adenovirus, parechovirus, or bocavirus. Thirty-eight (44%) of the 94 patients positive for adenovirus were ≤1 year of age (median age, 5.8 months). The majority (43.4%) of patients and healthy controls who tested positive for bocavirus were 13–24 months of age (median age, 19 months).

Seasonality

In patients with AGE, adenovirus was identified throughout the year, with the highest proportion of positive test results (17.2%) in June. Shedding of adenovirus in healthy controls was detected from January through May, with the highest proportion seen in April (Figure 2). In patients with AGE, sapovirus was detected

Table 4. Comparison of Clinical Characteristics Associated With Single Infections of Rotavirus, Norovirus, Sapovirus, Astrovirus, and Adenovirus in Children With Acute Gastroenteritis

Characteristic	Rotavirus[a] (n = 126)	Norovirus[a] (n = 125)	Sapovirus (n = 19)	Astrovirus (n = 16)	Adenovirus (n = 68)
Diarrhea duration					
0	6	20	37	6	13
0–95 h (1–4 d)	81	62	42	81	62
96–119 h (5 d)	7	6	0	6	15
≥120 h (≥6 d)	6	12	21	6	10
Diarrhea episodes per 24 h, maximum no.					
0	6	20	37	6	13
1–3	23	28	21	19	28
4–5	23	17	16	25	12
≥6	47	34	26	50	47
Vomiting duration					
0	6	5	11	31	15
0–23 h (1 d)	16	21	16	13	21
24–47 h (2 d)	21	21	37	38	28
≥48 h (≥3 d)	57	53	37	19	37
Vomiting episodes per 24 h, maximum no.					
0	6	5	11	31	15
1	8	8	26	6	13
2–4	28	33	26	38	41
≥5	58	54	37	25	31
Fever, °C					
≤37.0	23	55	47	63	51
37.1–38.3	36	19	21	19	24
38.4–38.8	16	6	5	13	6
≥38.9	26	21	26	6	19
Behavioral sign/symptom					
Normal	4	11	11	13	13
Less playful or fussy/irritable	23	41	42	56	44
More sleepy or seizure	72	48	47	31	43
Treatment					
None	26	31	47	38	28
Rehydration, no hospitalization	39	34	26	25	26
Hospitalization	35	31	32	38	46
Severity score[b]					
0 to ≤10	19	39	53	50	43
11 to ≤ 14	51	41	42	38	46
15 to 20	30	21	5	13	12

Data are percentage of subjects.

[a] Rotavirus and norovirus data from previous studies on the same sample collections were included [11, 12].

[b] Based on a modified 20-point scale of the Vesikari system [18]. Disease conditions are categorized as mild (score, 0–10), moderate (score, 11–14), or severe (score, 15–20).

throughout the year, with the highest prevalence (51.4%) from March through July (Figure 2), while in healthy controls sapovirus was detected from December through May. Astrovirus was identified in samples collected from November through May from both symptomatic and healthy children (Figure 2). Most parechovirus infections in patients (31.5%) and healthy controls (36.3%) were detected in July. Bocavirus was detected primarily from December through June in both patients and healthy controls. Because of the low incidence of aichivirus, a seasonal pattern for this virus could not be determined.

Typing

Genogroup I (GI) sapoviruses were detected in 63.3% of the sapovirus-positive patients, whereas GII sapoviruses were detected

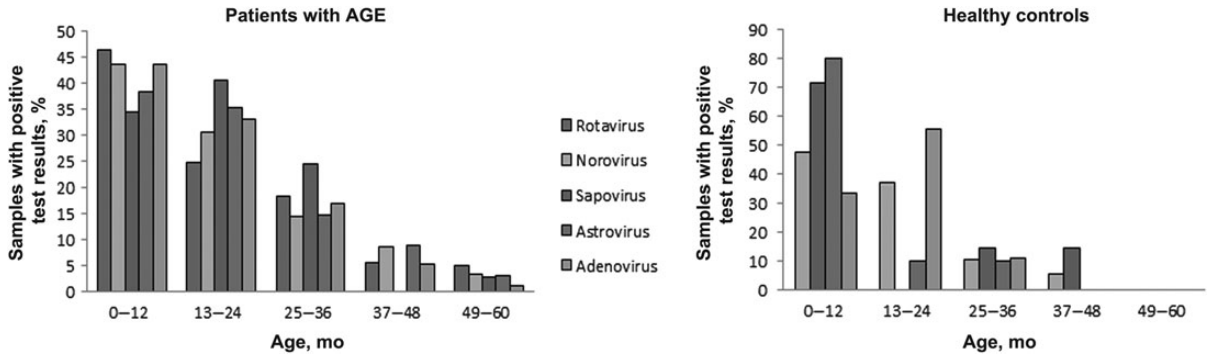

Figure 1. Age distribution (among samples positive for each virus) of 5 enteric viruses (rotavirus, norovirus, adenovirus, astrovirus, and sapovirus) in children <5 years of age with acute gastroenteritis (AGE) and healthy controls. Data on rotavirus and norovirus were previously published [11, 12] and are included for comparison.

in 75% of the sapovirus-positive healthy controls. The most prevalent sapovirus genotype was GI.1 (35.2%), followed by GI.2 (23.5%), GII.1 (17.6%), GII.UA (unassigned; 11.7%), GII.2 (5.8%), and GII.3 (5.8%; Figure 3*A*). Astrovirus type 1 was identified in 51.7% of patients, whereas 57.1% of astroviruses detected in the healthy controls were type 3. In addition, astrovirus type 2 (in 19.4%), type 4 (in 8.3%), and type 8 (in 2.7%) were identified (Figure 3*B*). All adenoviruses were type 41, and both aichivirus strains were genotype B (Figure 3*C*). Four different parechovirus types (1, 3, 4, and 5) and 3 bocavirus types (1, 2, and 3) were detected.

DISCUSSION

Our data highlight the important contribution of several enteric viruses to the etiology of AGE in young US children, identifying a virus in about two-thirds of them. With the decline of severe rotavirus disease following the introduction of rotavirus vaccines, noroviruses have emerged as the leading cause of medically attended childhood AGE, and these 2 viruses accounted for 40% of AGE cases [12]. Our data demonstrate that, in addition to norovirus and rotavirus, adenovirus was the most frequently detected virus in patients with AGE, followed by sapovirus and astrovirus. Overall, these 3 viruses were detected in 22.1% of patients with AGE. Despite their frequent detection in children with AGE, the presence of human parechovirus and bocavirus in equal or higher numbers in healthy controls does not support a causative role of these viruses in endemic AGE in children <5 years of age. Consistent with previous studies of the etiology of gastroenteritis in pediatric populations in the United States, most viruses, with the exception of adenovirus, showed a winter-spring seasonality [13, 31].

The prevalence of adenovirus in our study was higher (11.8%) than reported previously [32]. This could primarily be attributed to the high prevalence (27.5%) detected in children at the Rochester site, suggesting strong geographic variability of this virus. All adenovirus strains detected in this study were

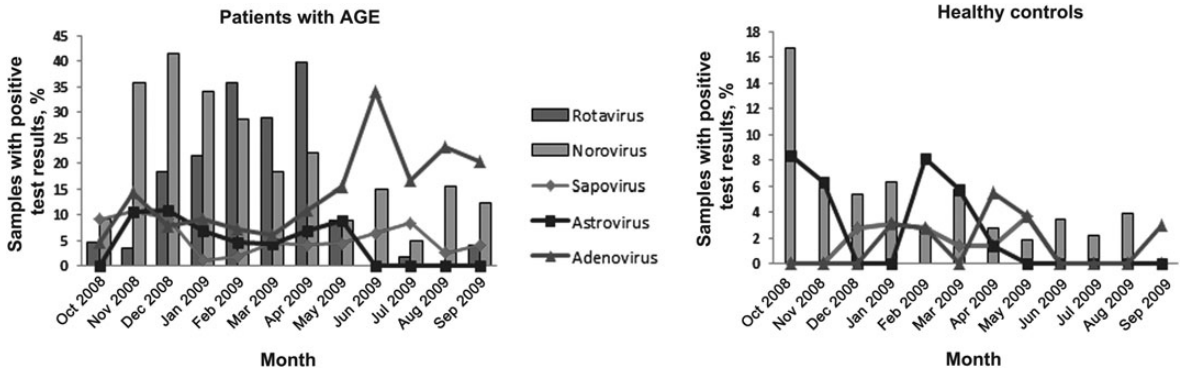

Figure 2. Seasonality of rotavirus, norovirus, adenovirus, astrovirus, and sapovirus infections in children <5 years of age in the United States from October 2008 through September 2009. Data on rotavirus and norovirus were previously published [11, 12] and are included for comparison. Abbreviation: AGE, acute gastroenteritis.

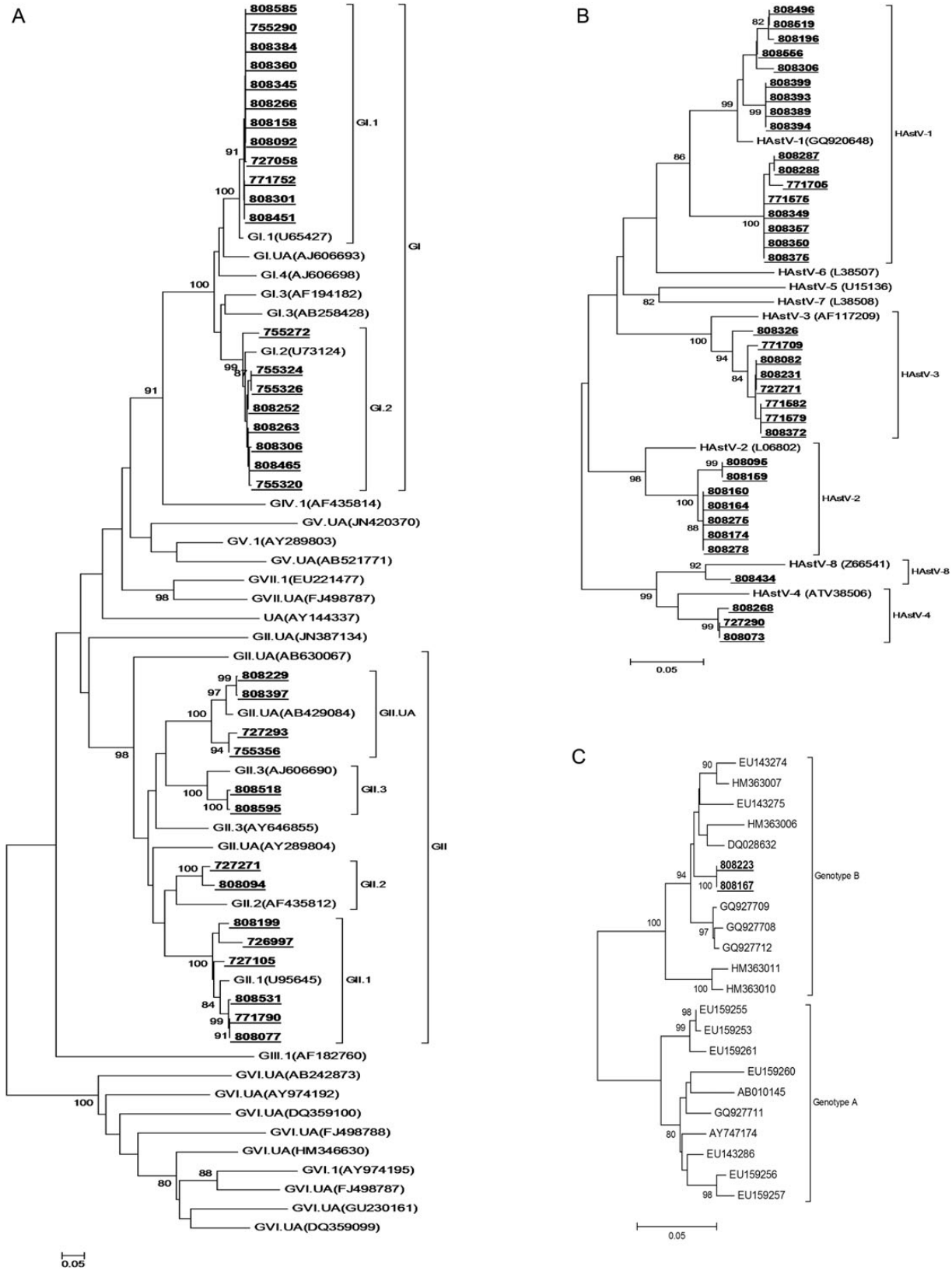

Figure 3. Phylogenetic trees were constructed using the neighbor-joining method from nucleotide sequences of partial capsid regions of sapovirus (*A*), astrovirus (*B*), and aichivirus (*C*) strains detected in this study and reference strains. The strains from this study are in bold and underlined, and the reference strains are indicated with their respective GenBank accession numbers. The numbers on the branches indicate bootstrap values. GII.UA denotes unassigned sapovirus GII genotype.

type 41. Of note, 72% of all adenovirus positive samples from Rochester were collected between March 2009 and September 2009. Interestingly, the positive adenovirus type 41 samples had identical sequences and were equally distributed among hospitalized children, patients in the ED, and outpatients, suggesting a summer epidemic due to this virus. Higher prevalence rates at the Rochester site, compared with the Nashville and Cincinnati sites, has been reported previously for rotavirus in 2007 [33] but not for norovirus [11].

The prevalence and clinical symptoms associated with astrovirus and sapovirus infections were in agreement with previous reports on these infections in hospitalized children [14, 15, 34]. Interestingly, vomiting and not diarrhea was the primary symptom in children testing positive for sapovirus, in contrast to reports from Europe, Asia, and South America [34–36]. Since diarrhea is generally an eligibility requirement for most AGE studies, the incidence of sapovirus infections may have been underestimated in other studies.

Human parechovirus and bocavirus are known to cause sepsis-like illness with meningitis and respiratory tract infections in children and young infants [37, 38]. These viruses are mostly transmitted via the fecal-oral route and are thought to be excreted in the feces without replication in the gastrointestinal tract [6]. However, frequent detection and the high prevalence of these viruses in fecal specimens of children with AGE worldwide have prompted questions about their possible association with viral gastroenteritis [5, 6, 39]. We found a higher or comparable prevalence of parechovirus and bocavirus in healthy controls, compared with children with AGE. Furthermore, most of the children with parechovirus also tested positive for ≥1 other AGE-associated virus. Thus, our data do not support the association of parechovirus or bocavirus with AGE in this study population. Similar results from studies from Southeast Asia and Europe also suggest that these viruses are not associated with AGE in children <5 years of age [40].

Aichivirus was first isolated in the stool from a patient with AGE in 1991 in Japan. Subsequently, aichivirus has been found in 0.5%–4.1% of young children and adults with AGE [4, 41–43]. To our knowledge, our study is the first to report the detection of aichivirus in US children, confirming a global distribution of this virus, albeit at a low prevalence. However, both aichivirus-positive specimens also tested positive for norovirus, making it difficult to associate AGE symptoms specifically to aichivirus.

Fairly high percentages (13.1%) of the patients with AGE were infected with multiple viruses. Some reports suggest that mixed infections may be responsible for more-severe diarrhea [44, 45]. Most mixed infections included norovirus, identified in 21% of fecal specimens from patients in this study population [12]. Combinations of norovirus-astrovirus and norovirus-adenovirus were most frequently identified as coinfections. Most viruses were detected in specimens from children <2 years of age, which is in agreement with earlier reports [6,9,46]. Interestingly, sapovirus and astrovirus were detected more commonly in patients aged 1–2 years, whereas in healthy controls these viruses were more frequent detected in children <1 year of age.

Adenovirus type 41, GI.1 sapovirus, and astrovirus type 1 were the most prevalent genotypes detected, confirming previous molecular characterization of these viruses [15–17, 34]. Parechovirus types 1, 3, 4, and 5 were found cocirculating, with parechovirus type 1 being the most frequently detected type, similar to other studies [5, 8, 38, 40]. Although diarrhea has been identified as a common symptom of parechovirus type 1 infection [47], our data do not confirm such an association.

While both bocavirus types (ie, 1 and 3) were identified in the majority of the patients and controls, we did not find an association with AGE. Bocavirus type 1 is typically associated with respiratory tract infections, whereas the association of bocavirus type 3 with human disease is currently unknown [7, 37]. All bocavirus type 2 infections in our study were identified only in patients as single infections, indicating their possible association with AGE, as described in previous studies [39]. Our study has several limitations. First, the surveillance was conducted in 3 US counties that may not be representative of the entire US population. Second, to assess whether the prevalence of adenovirus, astrovirus, and sapovirus in children <5 years varies year by year, testing for these viruses should be extended to several additional years.

In conclusion, 22.1% of AGE cases among hospitalized children, children who presented to EDs, and outpatient children <5 years of age in the United States were caused by adenovirus, astrovirus, and adenovirus. Together with rotavirus and norovirus, two-thirds of the children with AGE in our study had a virus detected. With rotavirus rates significantly reduced through the introduction of rotavirus vaccination in the United States [48], it will be important to establish the etiology of unexplained AGE to better understand and reduce pediatric AGE. Routine surveillance among children with AGE and healthy controls, using multiplex assays for detection of enteric pathogens [49], will further help in the assessment of the role of these agents in childhood diarrhea.

Notes

Acknowledgments. We thank the following individuals for their support and contributions: NVSN coinvestigators, James Chappell, MD, PhD (Vanderbilt University), Caroline B. Hall, MD, Geoffrey Weinberg, MD (University of Rochester, NY), and David Bernstein (Cincinnati Children's Hospital Medical Center); surveillance coordinators, Diane Kent, RN (Vanderbilt University), Gerry Loftus, PhD (University of Rochester, NY), and Jessica Schloemer (Cincinnati Children's Hospital Medical Center).

Disclaimer. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. This article received clearance through the appropriate channels at the Centers for Disease Control and Prevention prior to submission.

Financial support. This work was funded by the Centers for Disease Control and Prevention as part of the New Vaccine Surveillance Network.

Potential conflicts of interest. M. A. S. has received research funding from GlaxoSmithKline, receives funding from GlaxoSmithKline, and serves on the rotavirus advisory boards for Merck and GlaxoSmithKline. All other authors report no potential conflicts.

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

References

1. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet **2012**; 379:2151–61.
2. Jones TF, McMillian MB, Scallan E, et al. A population-based estimate of the substantial burden of diarrhoeal disease in the United States; FoodNet, 1996–2003. Epidemiol Infect **2007**; 135:293–301.
3. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States–unspecified agents. Emerg Infect Dis **2011**; 17:16–22.
4. Verma H, Chitambar SD, Gopalkrishna V. Circulation of Aichi virus genotype B strains in children with acute gastroenteritis in India. Epidemiol Infect **2011**; 139:1–5.
5. Benschop K, Thomas X, Serpenti C, Molenkamp R, Wolthers K. High prevalence of human Parechovirus (HPeV) genotypes in the Amsterdam region and identification of specific HPeV variants by direct genotyping of stool samples. J Clin Microbiol **2008**; 46:3965–70.
6. 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.
7. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog **2009**; 5:e1000391.
8. Zhang DL, Jin Y, Li DD, et al. Prevalence of human parechovirus in Chinese children hospitalized for acute gastroenteritis. Clin Microbiol Infect **2011**; 17:1563–9.
9. Medici MC, Martinelli M, Arcangeletti MC, et al. Epidemiological aspects of human rotavirus infection in children hospitalized with acute gastroenteritis in an area of northern Italy. Acta Biomed **2004**; 75:100–6.
10. Payne DC, Staat MA, Edwards KM, et al. Active, population-based surveillance for severe rotavirus gastroenteritis in children in the United States. Pediatrics **2008**; 122:1235–43.
11. Payne DC, Staat MA, Edwards KM, et al. Direct and indirect effects of rotavirus vaccination upon childhood hospitalizations in 3 US Counties, 2006–2009. Clin Infect Dis **2011**; 53:245–53.
12. Payne DC, Vinje J, Szilagyi PG, et al. Norovirus is a major cause of severe gastroenteritis among US children in the post-rotavirus vaccine era. N Engl J Med **2012**; 368:1121–30.
13. Zimmerman CM, Bresee JS, Parashar UD, Riggs TL, Holman RC, Glass RI. Cost of diarrhea-associated hospitalizations and outpatient visits in an insured population of young children in the United States. Pediatr Infect Dis J **2001**; 20:14–9.
14. Kotloff KL, Herrmann JE, Blacklow NR, et al. The frequency of astrovirus as a cause of diarrhea in Baltimore children. Pediatr Infect Dis J **1992**; 11:587–9.
15. Dennehy PH, Nelson SM, Spangenberger S, Noel JS, Monroe SS, Glass RI. A prospective case-control study of the role of astrovirus in acute diarrhea among hospitalized young children. J Infect Dis **2001**; 184:10–5.
16. Farkas T, Zhong WM, Jing Y, et al. Genetic diversity among sapoviruses. Arch Virol **2004**; 149:1309–23.
17. Zintz C, Bok K, Parada E, et al. Prevalence and genetic characterization of caliciviruses among children hospitalized for acute gastroenteritis in the United States. Infect Genet Evol **2005**; 5:281–90.
18. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand J Infect Dis **1990**; 22:259–67.
19. Vega E, Barclay L, Gregoricus N, Williams K, Lee D, Vinje J. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. Emerg Infect Dis **2011**; 17:1389–95.
20. Oka T, Katayama K, Hansman GS, et al. Detection of human sapovirus by real-time reverse transcription-polymerase chain reaction. J Med Virol **2006**; 78:1347–53.
21. Okada M, Yamashita Y, Oseto M, Shinozaki K. The detection of human sapoviruses with universal and genogroup-specific primers. Arch Virol **2006**; 151:2503–9.
22. Grant L, Vinje J, Parashar U, et al. Epidemiologic and clinical features of other enteric viruses associated with acute gastroenteritis in American Indian infants. J Pediatr **2012**; 161:110–5 e1.
23. Noel JS, Lee TW, Kurtz JB, Glass RI, Monroe SS. Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. J Clin Microbiol **1995**; 33:797–801.
24. Lyman WH, Walsh JF, Kotch JB, Weber DJ, Gunn E, Vinje J. Prospective study of etiologic agents of acute gastroenteritis outbreaks in child care centers. J Pediatr **2009**; 154:253–7.
25. Allard A, Albinsson B, Wadell G. Detection of adenoviruses in stools from healthy persons and patients with diarrhea by two-step polymerase chain reaction. J Med Virol **1992**; 37:149–57.
26. Nix WA, Maher K, Johansson ES, et al. Detection of all known parechoviruses by real-time PCR. J Clin Microbiol **2008**; 46:2519–24.
27. Nix WA, Maher K, Pallansch MA, Oberste MS. Parechovirus typing in clinical specimens by nested or semi-nested PCR coupled with sequencing. J Clin Virol **2010**; 48:202–7.
28. Kantola K, Sadeghi M, Antikainen J, et al. Real-time quantitative PCR detection of four human bocaviruses. J Clin Microbiol **2010**; 48:4044–50.
29. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **1994**; 22:4673–80.
30. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol **2007**; 24:1596–9.
31. Hall AJ, Rosenthal M, Gregoricus N, et al. Incidence of acute gastroenteritis and role of norovirus, Georgia, USA, 2004–2005. Emerg Infect Dis **2011**; 17:1381–8.
32. Rodriguez-Baez N, O'Brien R, Qiu SQ, Bass DM. Astrovirus, adenovirus, and rotavirus in hospitalized children: prevalence and association with gastroenteritis. J Pediatr Gastroenterol Nutr **2002**; 35:64–8.
33. Payne DC, Szilagyi PG, Staat MA, et al. Secular variation in United States rotavirus disease rates and serotypes: implications for assessing the rotavirus vaccination program. Pediatr Infect Dis J **2009**; 28:948–53.
34. Dey SK, Phan TG, Nguyen TA, et al. Prevalence of sapovirus infection among infants and children with acute gastroenteritis in Dhaka City, Bangladesh during 2004–2005. J Med Virol **2007**; 79:633–8.
35. Rockx B, De Wit M, Vennema H, et al. Natural history of human calicivirus infection: a prospective cohort study. Clin Infect Dis **2002**; 35:246–53.
36. Gonzalez GG, Liprandi F, Ludert JE. Molecular epidemiology of enteric viruses in children with sporadic gastroenteritis in Valencia, Venezuela. J Med Virol **2011**; 83:1972–82.
37. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A **2005**; 102:12891–6.
38. Harvala H, Simmonds P. Human parechoviruses: biology, epidemiology and clinical significance. J Clin Virol **2009**; 45:1–9.
39. 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.
40. Tapia G, Cinek O, Witsø E, et al. Longitudinal observation of parechovirus in stool samples from Norwegian infants. J Med Virol **2008**; 80: 1835–42.
41. Yamashita T, Kobayashi S, Sakac K, et al. Isolation of cytopathic small round viruses with BS-C-l cells from patients with gastroenteritis. J Infect Dis **1991**; 164:954–7.

42. Oh DY, Silva PA, Hauroeder B, Diedrich S, Cardoso DDP, Schreier E. Molecular characterization of the first Aichi viruses isolated in Europe and in South America. Arch Virol **2006**; 151:1199–206.
43. Pham NT, Khamrin P, Nguyen TA, et al. Isolation and molecular characterization of Aichi viruses from fecal specimens collected in Japan, Bangladesh, Thailand, and Vietnam. J Clin Microbiol **2007**; 45:2287–8.
44. Taylor MB, Marx FE, Grabow WO. Rotavirus, astrovirus and adenovirus associated with an outbreak of gastroenteritis in a South African child care centre. Epidemiol Infect **1997**; 119:227–30.
45. Roman E, Wilhelmi I, Colomina J, et al. Acute viral gastroenteritis: proportion and clinical relevance of multiple infections in Spanish children. J Med Microbiol **2003**; 52:435–40.
46. Rimoldi SG, Stefani F, Pagani C, et al. Epidemiological and clinical characteristics of pediatric gastroenteritis associated with new viral agents. Arch Virol **2011**; 156:1583–9.
47. Ito M, Yamashita T, Tsuzuki H, et al. Detection of human parechoviruses from clinical stool samples in Aichi, Japan. J Clin Microbiol **2010**; 48:2683–8.
48. Cortes JE, Curns AT, Tate JE, et al. Rotavirus vaccine and health care utilization for diarrhea in U.S. children. N Engl J Med **2011**; 365: 1108–17.
49. Liu J, Kibiki G, Maro V, et al. Multiplex reverse transcription PCR Luminex assay for detection and quantitation of viral agents of gastroenteritis. J Clin Virol **2011**; 50:308–13.