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ORIGINAL ARTICLE

Viral Etiology of Acute Gastroenteritis in <2-Year-Old US Children in the Post–Rotavirus Vaccine Era

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Background. The rotavirus disease burden has declined substantially since rotavirus vaccine was introduced in the United States in 2006. The aim of this study was to determine the viral etiology of acute gastroenteritis (AGE) in US children aged <2 years.

Methods. The New Vaccine Surveillance Network (NVSN) of geographically diverse US sites conducts active pediatric population-based surveillance in hospitals and emergency departments. Stool samples were collected from children aged <2 years with symptoms of AGE (n = 330) and age-matched healthy controls (HCs) (n = 272) between January and December 2012. Samples were tested by real-time reverse-transcriptase polymerase chain reaction assays {adenovirus (type 40 and 41), norovirus, parechovirus A, enterovirus, sapovirus, and astrovirus} and an enzyme immunoassay (rotavirus). All samples that tested positive were genotyped.

Results. Detection rates of pathogens in children with AGE versus those of HCs were, respectively, 23.0% versus 6.6% for norovirus ($P < .01$), 23.0% versus 16.0% for adenovirus ($P = .08$), 11.0% versus 16.0% for parechovirus A ($P = .09$), 11.0% versus 9.0% for enterovirus ($P = .34$), 7.0% versus 3.0% for sapovirus ($P = .07$), 3.0% versus 0.3% for astrovirus ($P = .01$), and 3.0% versus 0.4% for rotavirus ($P = .01$). A high prevalence of adenovirus was detected at 1 surveillance site (49.0% for children with AGE and 43.0% for HCs). Norovirus GII.4 New Orleans was the most frequently detected (33.0%) norovirus genotype. Codetection of >1 virus was more common in children with AGE (16.0%) than in HCs (10.0%) ($P = .03$).

Conclusions. Norovirus, astrovirus, sapovirus, and rotavirus were detected significantly more in children with AGE than in HCs, and norovirus was the leading AGE-causing pathogen in US children aged <2 years during the year 2012.

Keywords. acute gastroenteritis; children; codetection; epidemiology; genotyping; real-time PCR.

In the pre–rotavirus vaccine era in the United States, 40% to 50% of all diarrhea-associated hospitalizations of children younger than 5 years were attributable to rotavirus, which accounts for 60 000 to 70 000 hospitalizations and 500 000 health care visits per year [1]. Since the introduction of rotavirus vaccine in 2006, however, rotavirus-associated illness has decreased dramatically [2]. Recent analyses of inpatient data from children aged <5 years in 26 states found that rates of rotavirus-coded hospitalizations declined by 63% to 94% from 2008 to 2012 when compared to those of the pre–rotavirus vaccine period from 2000 to 2006 [3].

In addition to rotavirus, other established gastroenteritis viruses include norovirus, sapovirus, astrovirus, and enteric adenovirus types 40 and 41. In recent years, enterovirus and parechovirus A (previously known as human parechovirus) have been detected in stool samples from children with acute gastroenteritis (AGE) in different geographical locations around the world [4–7], although their association with AGE is not fully understood. Norovirus has become recently the leading cause of AGE in medically attended children, accounting for nearly 1 million health care visits annually [8]. Although most epidemiological studies conducted in the United States have focused on rotavirus and norovirus, relatively less information is available on other viruses that also are associated with a significant number of cases of AGE in children [4, 8–12].

To monitor the effectiveness of rotavirus vaccination in the United States, active population-based surveillance in several large medical institutions has been conducted by the New Vaccine Surveillance Network (NVSN) since 2006. The NVSN provides a unique opportunity to monitor the trends of established AGE-viruses, such as norovirus, and the emergence of potentially new viruses. In addition, the NVSN provides the ability to compare pathogen detection in stool specimens collected from children with AGE and healthy controls (HCs) to assess the role of these pathogens in AGE. The inclusion of geographically different sites enables determination of the regional prevalence of each AGE-causing virus. The aim

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of this study was to assess the epidemiology of established AGE-causing viruses, such as norovirus, enteric adenovirus (type 40 and 41), astrovirus, sapovirus, and rotavirus, and as well as enterovirus and parechovirus A in US children aged <2 years with AGE in the post–rotavirus vaccine era (January to December 2012). Virus detection in patients with AGE was compared with that in age-matched HCs to understand the association of these viruses with AGE.

METHODS

Study Design, Sample Collection, and Subject Selection

The participants in this study were a randomly selected subset of children aged <2 years enrolled through the NVSN sites between January and December 2012. The design and methods of the NVSN surveillance of AGE infections have been described already [8, 13]. For this specific study, we tested stool samples collected at Children's Mercy Hospital (Kansas City, Missouri), Vanderbilt University (Nashville, Tennessee), Seattle Children's Hospital (Seattle, Washington), and the University of Rochester (Rochester, New York).

Children were enrolled if they had symptoms of AGE for ≤10 days, if they were >14 days but <2 years of age, and regardless of whether they were hospitalized or seen in the emergency department (ED). AGE symptoms were defined as ≥3 diarrhea episodes within 24 hours, ≥1 vomiting episode within 24 hours, or both. Stool specimens were collected within 14 days of the date of ED visit or admission for AGE symptoms.

HCs were enrolled during well-child visits if they were between >14 days and <2 years of age and had no history of AGE within 14 days before enrollment and no history of respiratory illness (cough, congestion, runny nose, sore throat, or wheezing) within 3 days before enrollment. Any HC with a reported clinical immunodeficiency was not eligible to participate. The HCs in this study were frequency matched to the children with AGE on the basis of age and calendar month of enrollment. Stool specimens from the HCs were collected within 5 days after enrollment.

Interviews were conducted for each child with AGE and each HC. Demographic, epidemiologic, and clinical data, including age, sex, race, ethnicity, previous medical history, clinical signs and symptoms, date of onset of symptoms, and date of sample collection, were collected.

Children were enrolled via written informed consent from their parent(s) or legal guardian(s). This study was reviewed and approved by the institutional review boards of each participating site and the Centers for Disease Control and Prevention.

Sample Preparation and Total Nucleic Acid Extraction

After participant enrollment, stool samples were prepared by 2 different procedures. When possible, raw stool samples were collected directly from diapers, and several aliquots were made and stored at −80°C for later use. If the amount of stool was insufficient for direct collection, then a small area of the soiled diaper was cut and flushed with 10 mL of Earle's balanced salt solution (EBSS) using a 10-mL syringe. Several aliquots were made and stored at −80°C for later use.

Frozen aliquots of stool samples were thawed, and 100 µL of liquid stool was added to a tube of 400 µL of nuclease-free distilled water. The tube was vortexed thoroughly and centrifuged. Supernatant was collected in a separate tube and stored at −80°C. For the stool samples flushed with EBSS, 180 µL was used directly for nucleic acid extraction as described later. If the stool took the shape of the container and was difficult to pipette, a pea-sized amount of the stool was removed using a disposable smartSpatula (VWR, Radnor, Pennsylvania), mixed into 500 µL of distilled water, and processed as noted earlier. Aliquoted supernatant (180 µL) was mixed with 20 µL of bacteriophage MS2 (internal control) and used for total nucleic acid extraction by using the NucliSENS easyMag automated extraction system according to manufacturer instructions and eluted in 55 µL of elution buffer (bioMérieux, Inc, Durham, North Carolina). After extraction, 2 aliquots were stored at −20°C until further testing. RNA extraction for rotavirus genotyping was performed as described previously [10].

Real-Time Polymerase Chain Reaction Assays, Enzyme Immunoassay, and Genotyping

Samples were tested for adenovirus (type 40 and 41), genogroup I (GI) and GII norovirus, sapovirus, astrovirus, parechovirus A, and enterovirus by real-time reverse-transcription polymerase chain reaction (RT-PCR) assays, as described previously [4, 14–16]. We used an enzyme immunoassay to detect rotavirus (Premier Rotaclone, Meridian Bioscience, Inc, Cincinnati, Ohio). Samples with a positive test result were genotyped further by sequencing partial regions of the capsid gene (region C) (norovirus), hexon (adenovirus), and ORF2 (sapovirus and astrovirus) according to published methods [4]. Parechovirus A– and enterovirus-positive samples were typed by sequencing the VP1 region [17, 18]. VP4 and VP7 genotyping and sequencing of rotavirus-positive samples were performed as described previously [14].

All nucleic acid extractions and real-time RT-PCR assays were performed at Children's Mercy Hospital. Rotavirus testing by enzyme immunoassay was conducted at each participating site. All genotyping was performed at the Centers for Disease Control and Prevention.

Statistical Analysis

Statistical differences between the AGE and HC groups for each virus were determined by the Fisher exact test. *P* values of <.05 were considered statistically significant.

RESULTS

Overall Prevalence of AGE-Causing Viruses

Stool samples collected from 602 participants between January 2012 and December 2012 from 4 participating sites were included for testing in this study (Figure 1). Among these samples, 330 were from children with AGE and 272 were from HCs. Of the 330 children with AGE, 64 (19%) were hospitalized, and the remainder were seen in the ED and discharged. Overall, at least 1 virus was detected in 315 (52%) of the 602 stool samples. More stool samples from patients with AGE were positive for at least 1 of the viruses tested (63% [207 of 330]) than were stool samples from HCs (40% [108 of 272]) ($P = .001$). Approximately 24% (143 of 602) of the samples were stool extractions with EBSS. Among them, 34% (113 of 302) were from children with AGE and 11% (30 of 272) were from HCs. However, the rates of positivity were similar in the EBBS extraction group and the overall AGE group (63.0% vs 61.0%, respectively) and HC group (40.0% vs 30.0%, respectively). Rates for individual viruses among children with AGE versus HCs are shown in Table 1. Important to note is that the virus with the lowest detection rate in children with AGE was rotavirus (3%). Also important is that norovirus was detected consistently in samples from all 4 sites, and the highest detection rate was found in Seattle (29%). Approximately half of the children in both the AGE (49%) and HC (43%) groups from Nashville tested positive for adenovirus. More cases of parechovirus A were detected in HCs (16%) than in the children with AGE (11%) ($P = .09$). Among hospitalized children with AGE, 48% (31 of 64) tested positive for at least 1 virus. Adenovirus was the virus detected most frequently (20% [13 of 64]) in these hospitalized participants, followed by norovirus (12%), parechovirus A (11%), sapovirus (6%), enterovirus (5%), astrovirus (3%), and rotavirus (1%).

Overall, the median cycle threshold (Ct) was significantly lower in stool specimens from children with AGE than in those from HCs, which suggests a higher viral load in children with AGE caused by norovirus (26.2 vs 30.7, respectively; $P = .0026$), adenovirus (29.3 vs 33.5, respectively; $P = .0001$), or sapovirus (27.7 vs 33.6, respectively; $P = .0125$) (Figure 2). Similar trends of viral load were observed between single-virus detections in children with AGE and HCs, most prominently for adenovirus (data not shown). The median Ct value of single adenovirus detection in children with AGE was 16.8 [interquartile range (IQR) 12.7–32.1], which is much lower than that in HCs (median Ct, 33.49 [IQR 31.9–36.0]). Samples extracted by EBSS tended to have significantly higher Ct values than did raw stool for norovirus (median Ct, 29.5 vs 25.15, respectively) or adenovirus (median Ct, 31.8 vs 21.8, respectively) ($P \leq .05$).

Single Detection Versus Codetection

Among 330 stool samples from children with AGE, a single virus was detected in 46% (153 of 330) of them; more than 1 virus was detected in 16% (54 of 330) of them (Table 2). In 272 HCs, 29% (80 of 272) tested positive for a single virus, and 10% (28 of 272) had more than 1 virus. The detection rate for single and multiple virus-positive samples was significantly higher in children with AGE than in HCs ($P = .0001$ and .0321 for children with a single or multiple virus-positive sample, respectively). The top 2 viruses with a single detected infection were norovirus (33% [50 of 153]) and adenovirus (29% [44 of 153]) in children with AGE and parechovirus A (35% [28 of 80]) and adenovirus (30% [24 of 80]) in HCs.

Codetection of more than 1 virus was found in 54 children with AGE and 28 HCs (Table 2). Adenovirus was the most frequently detected virus in codetections in both the children with AGE (27.0% [31 of 114]) and the HCs (32.0% [19 of 59]). Codetection of both norovirus and adenovirus (22% [12 of 54]) was most frequent among children with AGE (Table 3). In contrast, the combination of adenovirus and enterovirus (25% [7 of 28]) was detected most frequently in HCs.

Age Distribution of Viruses Among Children With AGE and HCs

Among the 4 age groups (0–6, 7–12, 13–18, and 19–23 months), norovirus was detected most frequently (74% [70 of 94]) in

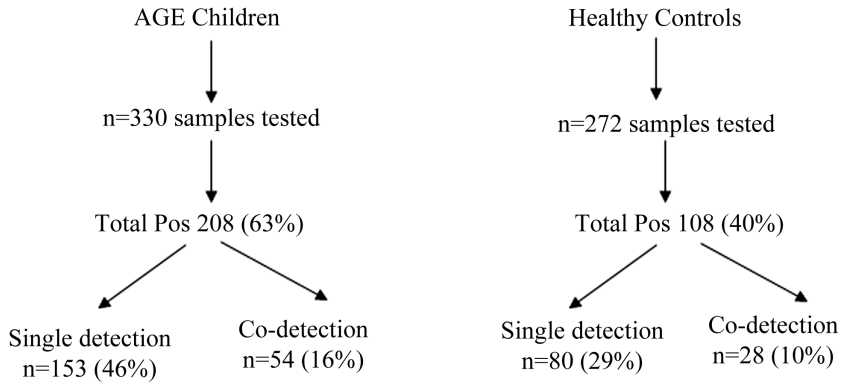


Figure 1. Flowchart of number of samples tested in children with acute gastroenteritis (AGE) and healthy controls. Abbreviation: Pos, positive.

Table 1. Rates of Positivity for Norovirus, Adenovirus, Parechovirus A, Enterovirus, Sapovirus, Astrovirus, and Rotavirus in Children With Acute Gastroenteritis and HCs: 2012

	Positivity Rate (% [n]) According to Location										
	Kansas City		Seattle		Nashville		Rochester		Total		
Virus	AGE (n = 101)	HCs (n = 108)	AGE (n = 85)	HCs (n = 44)	AGE (n = 91)	HCs (n = 88)	AGE (n = 53)	HCs (n = 32)	AGE (n = 330)	HCs (n = 272)	*P*
Norovirus	17.8 (18)	9.2 (10)	29.4 (25)	2.2 (1)	26.4 (24)	5.6 (5)	17.0 (9)	6.2 (2)	23.0 (76)	6.7 (18)	.0001
Adenovirus	17.8 (18)	4.6 (5)	10.5 (9)	0 (0)	49.4 (46)	43.1 (38)	3.7 (2)	3.1 (1)	22.7 (75)	16.1 (44)	.08
Parechovirus A	9.9 (10)	14.8 (16)	9.4 (8)	13.6 (6)	12.1 (11)	19.3 (17)	15.1 (8)	15.6 (5)	11.2 (37)	16.1 (44)	.09
Enterovirus	12.9 (13)	9.2 (10)	8.23 (7)	0 (0)	15.4 (14)	14.7 (13)	5.6 (3)	3.1 (1)	11.2 (37)	8.8 (24)	.34
Sapovirus	9.9 (10)	3.7 (4)	2.3 (2)	0 (0)	6.6 (6)	2.3 (2)	7.5 (4)	9.3 (3)	6.6 (22)	3.3 (9)	.07
Astrovirus	3.9 (4)	0.9 (1)	4.7 (4)	0 (0)	0 (0)	0 (0)	3.7 (2)	0 (0)	3.03 (10)	0.4 (1)	.01
Rotavirus	1.0 (1)	0.9 (1[a])	5.8 (5)	0 (0)	4.3 (4)	0 (0)	0 (0)	0 (0)	3.0 (10)	0.4 (1[a])	.01

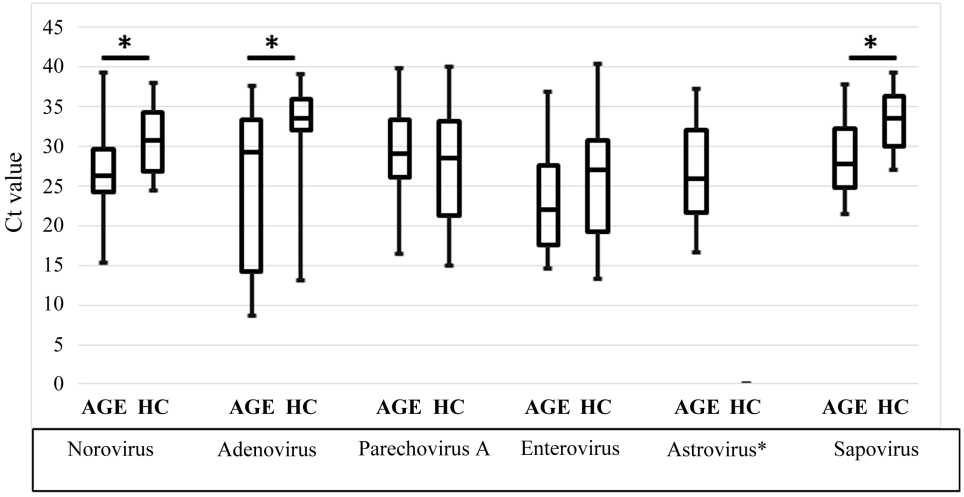
Abbreviations: AGE, children with acute gastroenteritis; HC, healthy control.
[a]Negative according to real-time reverse-transcription polymerase chain reaction.

7- to 18-month-old children with AGE (Figure 3). The median age for norovirus-positive children was 12 months (IQR, 8–16 months) and was similar to that for adenovirus-positive children (median, 11 months [IQR, 5–15 months]). We found no differences among the age groups in the detection of adenovirus, parechovirus A, or enterovirus in children with AGE (Figure 3A). Interesting to note is that the rates of astrovirus and sapovirus detection increased with increased age (medians, 16 months [IQR, 10–21 months] and 14 months [IQR, 7–17 months], respectively).

In HCs, parechovirus A was detected most frequently in older children (aged 19–23 months) (Figure 3B). In contrast, adenovirus was detected more frequently in 0- to 18-month-old children with AGE (median, 9 months [IQR, 3.5–13 months]). The median age of children with an enterovirus-positive stool sample was 10 months (IQR, 6–13 months), and this virus was detected more frequently in children between 7 and 23 months of age.

Genotyping

Among 94 norovirus-positive samples (76 from children with AGE, 18 from HCs), sequences were obtained from 85 (90.4%) samples (70 from children with AGE, 15 from HCs). Overall, GII viruses were more prevalent (92% [78 of 85]) than GI viruses (8% [7 of 85]). GII.4 New Orleans was the most common genotype in children with AGE (33%) and in HCs (33%) (Table 4). Other genotypes detected were GII.6 (9% in children with AGE, 7% in HCs), GII.3 (9% in children with AGE only), GII.1 (7%



*Statistically significant.
**Only one HC sample was positive (Ct 36.9) and box plot could not be plotted.

Figure 2. Cycle threshold (Ct) value for norovirus, adenovirus, parechovirus A, enterovirus, astrovirus, and sapovirus in children with acute gastroenteritis (AGE) and healthy controls (HC). *, statistically significant; **, only 1 sample from an HC tested positive (Ct, 36.9) and the box plot could not be plotted.

Table 2. Viral Detection in Children With AGE and HCs

	Children With AGE (n = 330)		HCs (n = 272)	
Virus	Single Detection (% [n]) (n = 153)	Codetection (% [n]) (n = 114[a])	Single Detection (% [n]) (n = 80)	Codetection (% [n]) (n = 59[b])
Norovirus	33.0 (50)	23.0 (26)	15.0 (12)	10.0 (6)
Adenovirus	29.0 (44)	27.0 (31)	30.0 (24)	32.0 (19)
Parechovirus A	11.0 (17)	17.0 (20)	35.0 (28)	27.0 (16)
Enterovirus	12.0 (19)	16.0 (18)	12.0 (10)	23.0 (14)
Sapovirus	7.0 (11)	10.0 (11)	6.0 (5)	7.0 (4)
Astrovirus	5.0 (8)	2.0 (2)	1.0 (1)	0 (0)
Rotavirus	3.0 (4)	5.0 (6)	0 (0)	0 (0)

Abbreviations: AGE, acute gastroenteritis; HC, healthy control.
[a]Total number of viral targets detected from 54 children with AGE.
[b]Total number of viral targets detected from 28 HCs.

in children with AGE and HCs), and GII.4 Den Haag (7% in children with AGE only). Among rotavirus-positive samples, G12P[8] was the most prevalent strain (90.0% [9 of 10]).

Of all adenovirus-positive samples, type 41 was the most common (89% [48 of 54]) circulating strain in both children with AGE and HCs. Parechovirus A1 was detected in 71% of children with AGE and 61% of HCs. Other parechovirus A genotypes were parechovirus A6 (8% in children with AGE) and parechovirus A14 (7% in HCs).

Among enterovirus-positive samples, coxsackievirus A6 (CVA6) was detected in 27% of children with AGE, whereas CVA2, CVA6, and echovirus 25 (E25) each were present in 15% of HCs. CVA5 was detected in 9% of children with AGE. A small number of other enteroviruses were detected in both groups (CVA10, CVA16, E3, E6, E14, and E21).

DISCUSSION

Norovirus was the leading viral agent detected across all 4 sites in our study between January and December 2012. Norovirus was observed in one-third (33%) of the children with AGE in whom a single virus was detected and among 23% (range, 18%–29%) of total detections. Adenovirus was also an important cause of AGE in these children; however, its total detection rates varied widely (average, 23% [range, 4%–49%]), likely as a result of sporadic outbreaks. Only 3.0% of the children with AGE tested positive for rotavirus in our 1-year study period. Important to note is that almost two-thirds (63%) of the children with AGE who either sought medical attention in the ED or required hospitalization were infected with at least 1 AGE-causing virus.

Table 3. Distribution of Viral Codetections in Children With AGE and HCs

Codetected Viruses	Children With AGE (n = 54) (% [n])	HCs (n = 28) (% [n])
Adenovirus and norovirus	22.0 (12)	7.0 (2)
Adenovirus and enterovirus	13.0 (7)	25.0 (7)
Parechovirus A and norovirus	9.0 (5)	7.0 (2)
Adenovirus and parechovirus A	7.0 (4)	18.0 (5)
Enterovirus and norovirus	7.0 (4)	4.0 (1)
Parechovirus A and sapovirus	5.0 (3)	7.0 (2)
Adenovirus, parechovirus A, and norovirus	4.0 (2)	0 (0)
Adenovirus, parechovirus A, and sapovirus	2.0 (1)	4.0 (1)
Adenovirus, parechovirus A, and enterovirus	0 (0)	11.0 (3)
Others	30.0 (16)	18.0 (5)

Abbreviations: AGE, acute gastroenteritis; HC, healthy control.

The overall prevalence of norovirus among patients with AGE (23% of both single detections and codetections) corresponds well with previous NVSN data [8]. Among the norovirus test–positive cases, 33% of children with AGE were infected with GII.4 New Orleans and 18% with GII.4 Sydney, whereas the virus detected in 5 children with AGE was typed as GII.4 Den Haag. In a previous study, GII.4 Den Haag virus was the predominant (71%) GII.4 variant in 2009, but it was replaced by GII.4 New Orleans in 2010 [8]. Our study results confirm a diverse cocirculation of norovirus genotypes, but GII.4 viruses contributed most of the infections in US children. Continuous monitoring of norovirus genotype distribution over time will be critical for norovirus vaccine development.

We observed that rotavirus detections can be rare in the post–rotavirus vaccine era, particularly during the winters of even-numbered years in the era's biennial transmission pattern [19]. Only 3% of the children with AGE in whom a single virus was detected were rotavirus test positive. Of the 11 total samples that tested positive for rotavirus by the enzyme immunoassay, 1 sample from an HC was found later to be negative according to real-time RT-PCR. Nine of the 10 samples, all from children with AGE, were genotyped as G12P[8], the most prevalent strain circulating in 2012 [14].

The rate of adenovirus detection in our patients with AGE in whom only a single virus was detected (29%) is much higher than that reported in previous studies (range, 2%–12%) [4, 20]. However, nearly half of our adenovirus detections among patients with AGE were from just 1 site (Nashville), which

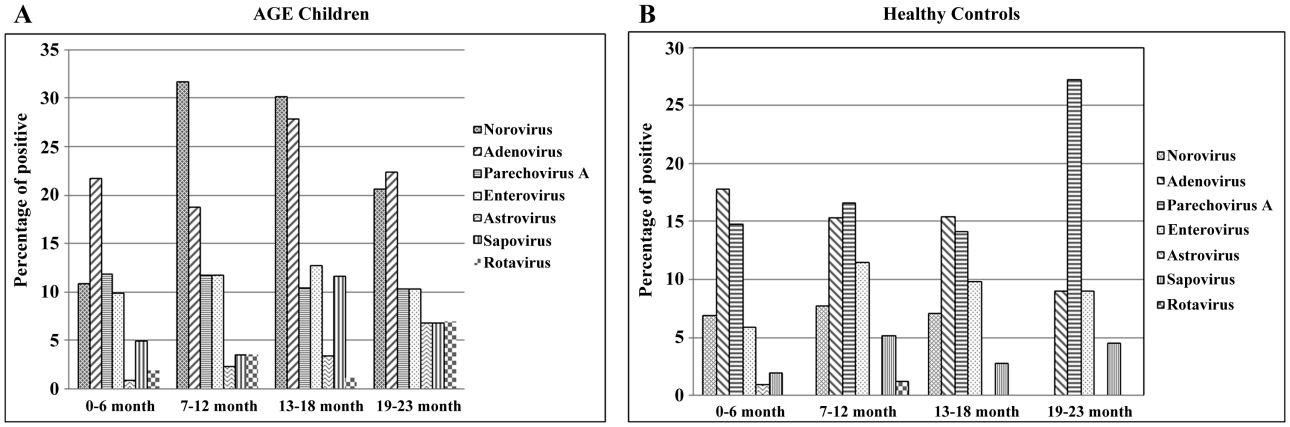


Figure 3. Age distributions of norovirus, adenovirus, parechovirus A, enterovirus, astrovirus, sapovirus, and rotavirus in children acute gastroenteritis (AGE) and healthy controls.

likely indicates a previously unrecognized adenovirus outbreak in this geographical area. Further supporting this hypothesis is that adenovirus was detected also in a large proportion (43%) of Nashville's HCs. Because 95% (36 of 38) of the adenoviruses in the HCs had a high Ct value of >30, our findings potentially indicate viral shedding among a large wave of recent but resolved infections. Approximately 90% of these detections in children with AGE and HCs shared enteric adenovirus type 41. Adenovirus outbreaks restricted to small geographical areas have been recorded. In a multisite study, 60% of the children with AGE who tested positive for adenovirus were from Rochester, New York, in 2008 to 2009 [4].

Despite the relatively high number (11%) of parechovirus A detections in children with AGE, we could not confirm that this virus is associated with AGE, because it was detected more frequently in the HCs (16%). Similar to observations in previous studies, the detection of parechovirus A in at least some stool samples might represent asymptomatic shedding, given the high rates of detection in both the AGE and HC samples [4, 6, 7].

The virus codetection rates in our study were 16% for children with AGE and 10% for HCs and were higher than those in other studies of US children but lower than those in studies conducted in developing countries [4, 21, 22]. In general, codetection rates are lower in developed countries than in developing countries, mostly as a result of better hygiene, sanitation, access to potable water, and overall living conditions in the developed countries. In a previous study conducted in US children in 2008 to 2009, the rates of codetection were 13% and 1% for children with AGE and HCs, respectively [4]. In contrast, significantly higher rates of codetection were reported in poor and/or developing countries. In a recent Global Enteric Multicenter Study (GEMS) conducted in countries with a lower socioeconomic status (sub-Saharan Africa and South Asian countries), 45% of children aged <5 years with diarrhea (cases) and 31% of children without diarrhea (controls) were infected with 2 or more pathogens [22]. In our study, we found that the rate of codetection in HCs (10%) was higher than that described previously (1%), primarily because of the higher rates of detection of adenovirus and parechovirus A in HCs.

Asymptomatic shedding of viral AGE-causing pathogens from participants without clinical AGE symptoms has long been documented [23, 24] and has become more relevant in the era of molecular detection methods, which are much more

Table 4. Genotype Distribution of the 2 Most Prevalent Strains for Each Reported Virus in Children With AGE and HCs

	Children With AGE (n = 191) (% [n/N])		HCs (n = 89) (% [n/N])	
Virus	Most Prevalent	Second Most Prevalent	Most Prevalent	Second Most Prevalent
Norovirus	GII.4 New Orleans (32.8 [23/70])	GII.4 Sydney (18.6 [13/70])	GII.4 New Orleans (33.3 [5/15])	GI.3B (20.0 [3/15])
Adenovirus	Adenovirus 41 (89.0 [33/37])	Adenovirus C/Ad2 (11.0 [4/37])	Adenovirus 41 (88.0, 15/17])	Adenovirus C/Ad2 (12.0 [2/17])
Parechovirus A	Par-A1 (71.0 [17/24])	Par-A3 (21.0 [5/24])	Par-A1 (61.0 [17/28])	Par-A3 (25.0 [7/28])
Enterovirus	CVA6 (27.0 [9/33])	E-11 (15.0 [5/33])	CVA2, 6, 16, E25 (15.0 [3/20])	EV-71 (10.0 [2/20])
Sapovirus	SaV GI.1 (29.4 [5/17])	SaV GI.2, SaV GII.1 (23.5 [4/17])	SaV GI.1 (62.5 [5/8])	SaV GII.2 (25.0 [2/8])
Astrovirus	AsV-1 (50.0 [5/10])	AsV-5 (30.0 [3/10])	AsV-3 (100.0 [1/1])	NA
Rotavirus	G12P[8] (90.0 [9/10])	G2P[4] (10.0 [1/10])	NA	NA

Abbreviations: AGE, acute gastroenteritis; HC, healthy control; NA, not applicable.

sensitive than traditional viral culture and rapid antigen tests. Because the detection of viral AGE-causing pathogens by PCR does not uniformly predict causality of disease, many groups have tried to establish a PCR Ct cutoff value that correlates with symptomatic versus asymptomatic infection. Such Ct cutoff values have been described for *Shigella* spp, enterotoxigenic *Escherichia coli* (ETEC), rotavirus, norovirus, astrovirus, and sapovirus [25–28]. All of those studies concluded that a Ct cutoff value might improve the differential diagnosis of infection with the specific pathogen. Although we did not determine the Ct cutoff value for each pathogen in this study, we found that, on average, HCs who were asymptomatic but had a detectable AGE-causing virus had a higher Ct (lower viral load) than children who had symptomatic AGE.

Our study had several limitations. First, we tested all samples collected in 1 calendar year rather than over a viral gastroenteritis winter season. By choosing 1 calendar year, we might have missed viruses that did not have an annual circulation pattern. Also, only randomly selected instead of all stool samples from each of the participating sites collected in 2012 were tested because of budget limitations. Another limitation is that we tested for only the most common viral enteric pathogens, not for other microbial AGE-causing pathogens or toxins secreted by pathogens. We were unable to find an etiologic agent in 37% of the stool samples from children with AGE. Other pathogens such as bacteria, parasites, toxins, and other AGE-causing viruses for which we did not test in our study might have been responsible for AGE in the virus-negative children with AGE. Last, unlike for all other AGE-causing viruses, rotavirus was detected by an antigen-based immunoassay, which is generally less sensitive (85% sensitivity reported for Rotaclone EIA assay) than molecular-based assays and might have led to a lower detection rate [29].

In summary, our data suggest that norovirus has emerged as the leading cause of AGE in US children aged <2 years. Norovirus, astrovirus, sapovirus, and rotavirus were detected at significantly higher frequencies in children with AGE than in HCs. Adenovirus outbreaks can occur sporadically in a particular geographic area in children with AGE and shed in otherwise healthy asymptomatic children. Continued active population-based surveillance for AGE in children will contribute to our understanding of emerging trends and provide important information for future vaccine development.

Notes

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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