

Viral Gastroenteritis in Children in Colorado 2006–2009

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Acute gastroenteritis accounts for a significant burden of medically attended illness in children under the age of five. For this study, four multiplex reverse transcription PCR assays were used to determine the incidence of adenovirus, astrovirus, coronavirus, norovirus GI and GII, rotavirus, and sapovirus in stool samples submitted for viral electron microscopy (EM) to the Children's Hospital Colorado. Of 1105 stool samples available, viral RNA/DNA was detected in 247 (26.2%) of 941 pediatric samples (median age = 2.97 years, 54% male) with 28 (3.0%) positive for more than one virus. Adenovirus, astrovirus, norovirus GI, norovirus GII, rotavirus, and sapovirus were detected in 95 (10.0%), 33 (3.5%), 8 (0.9%), 90 (9.6%), 49 (5.2%), and 2 (0.2%) of the pediatric samples, respectively. No coronaviruses were identified. Sequencing of norovirus positive samples indicated an outbreak of norovirus strain GII.4 in 2006 with evidence of numerous circulating strains. Multiple samples from the same immunocompromised patients demonstrated symptomatic shedding of norovirus for up to 32 weeks and astrovirus for 12 weeks. RT-PCR detected 99 of 111 (89%) adenovirus-positive samples versus 12 (11%) by EM, and 186 of 192 (97%) sapovirus/astrovirus/norovirus-positive samples versus 21 (11%) by EM. Noroviruses and adenoviruses are common causes of gastroenteritis in children. Immunocompromised patients can be infected with multiple viruses and shed viruses in their stools for prolonged periods. This data support the superiority of RT-PCR compared to EM for diagnosis of viral gastroenteritis. **J. Med. Virol.** 87:931–939, 2015. © 2015 Wiley Periodicals, Inc.

KEY WORDS: norovirus; pediatric; RT-PCR; epidemiology; immunocompromised; shedding

INTRODUCTION

Acute gastroenteritis in children, particularly under the age of five, remains a major cause of morbidity and mortality worldwide, with an estimated incidence of 2.5 billion cases annually [Unicef WHO, 2009; Gautam et al., 2013]. Acute gastroenteritis is the second leading cause of mortality in study patients outside the neonatal period and accounts for an estimated 1.5 million deaths each year. In the United States, acute gastroenteritis in children under five is responsible for approximately 300 deaths, 200,000 hospitalizations, and 1.5 million outpatient visits, an estimated \$250 million in direct medical costs, and \$1 billion in indirect medical costs [de Bruin et al., 2006; Churgay and Aftab, 2012].

In children, viruses are the major causes of acute gastroenteritis [Parashar et al., 2003; Terletskaia-Ladwig et al., 2007]. Rotavirus, adenovirus, astrovirus, norovirus, and sapovirus, have all been established as etiological agents of acute gastroenteritis in children [Hammond et al., 1981; Wilhelmi et al., 2003; Claas et al., 2005; Colomba et al., 2006; Unicef WHO, 2009; Churgay and Aftab, 2012; Chhabra et al., 2013; Payne et al., 2013]. Additionally, some clinical studies have suggested that coronaviruses may be a cause of acute gastroenteritis [Payne et al., 1986; van Maarseveen et al., 2010; Jevšnik

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et al., 2013]. Clinical data indicates that severe acute respiratory syndrome coronavirus (SARS-CoV) caused significant diarrhea [Booth et al., 2003; Choi et al., 2003; Hsu et al., 2003; Peiris et al., 2003; Hsueh et al., 2004; Grant et al., 2012]. However, the true significance of endemic coronaviruses as gastrointestinal pathogens has not been fully elucidated [Trujillo et al., 2006; Dominguez et al., 2009; Esper et al., 2010; Jevšnik et al., 2013].

Electron microscopy (EM) has been the gold standard diagnostic method for acute gastroenteritis, but this method requires a high concentration of viral particles per gram of stool, is labor-intensive, [Kuypers et al., 2007; Wei et al., 2013] and is now infrequently used. Many laboratories use commercially available immunoassays for the diagnosis of rotavirus, norovirus, and adenovirus diseases; however, these tests are limited by their ability to detect a single viral pathogen at a time, poor sensitivity and specificity, and limited availability outside suspected outbreaks [de Bruin et al., 2006; Terletskaia-Ladwig et al., 2007; Harris et al., 2009; Mans et al., 2010; Chhabra et al., 2013; Gautam et al., 2013]. Laboratories have been transitioning toward molecular-based techniques like RT-PCR for detection of etiologic viruses in patients with diarrheal illness.

The primary objective of this study was to determine the epidemiology of six common known enteric viruses (adenovirus, astrovirus, norovirus GI and GII, rotavirus, and sapovirus) as well as assess the role of the known endemic human coronaviruses in acute gastroenteritis in children. The secondary objective of this study was to compare the sensitivities of electron microscopy and RT-PCR for the diagnosis of viral acute gastroenteritis. This is the first hospital-based, multi-year, longitudinal study evaluating multiple viral etiologies of acute gastroenteritis in pediatric patients in the United States.

MATERIALS AND METHODS

Study Patients

All stool samples from September 2006 to June 2009 submitted for viral diagnostic testing by electron microscopy (EM) at Children's Hospital Colorado (CHCO) in Aurora, CO were diluted in sterile deionized water, vortexed, centrifuged, further diluted to 2.5% v/v of original specimen, and archived at -70°C . For EM, samples were concentrated by ultracentrifugation for 30 min at 22 psi, using an Airfuge and EM90 rotor (Beckman Coulter, Fullerton CA) [Hammond et al., 1981; Payne et al., 2013] negatively stained and scanned at $25,000\times$ magnification for virus particles. Use of the banked specimens and clinical data was approved by the Colorado Multiple Institutional Review Board.

DNA/RNA Extraction From Fecal Specimens

Nucleic acids from each sample were extracted on a Qiagen Biorobot EZ1 using the EZ1 viral RNA mini

kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, was eluted in RNase-free water, and stored at -80°C . Samples were divided into groups of 6 samples, and 10 microliters of each sample was combined. Each 60 microliter pool was stored at -80°C .

PCR and Sequencing

Four separate multiplex RT-PCR assays, based on modifications of previously published assays, were used to screen for the following viruses: adenovirus (family: *Adenoviridae*, genus: *Mastadenovirus*, species: *Human adenovirus A-F*) [Claas et al., 2005; Payne et al., 2013] and rotavirus (family: *Reoviridae*, subfamily: *Sedoreovirinae*, genus: *Rotavirus*, species: *rotavirus A*) [van Maarseveen et al., 2010; Sánchez-Fauquier et al., 2011], astrovirus (family: *Astroviridae*, genus: *Mamastrovirus*) and sapovirus (family: *Caliciviridae*, genus: *Sapovirus*, species: *Sapporovirus*) [Hsueh et al., 2004; De Benedictis et al., 2011; Grant et al., 2012], norovirus groups I and II (family: *Caliciviridae*, genus: *Norovirus*, species: *Norwalk virus*, genogroups GI and GII) [Trujillo et al., 2006; Finkbeiner et al., 2008], and the four known endemic human coronaviruses (order: *Nidovirales*, family: *Coronaviridae*, genus: *Alphacoronavirus* species: *human coronavirus 229E* and *NL63*; genus: *Betacoronavirus*, species: *human coronavirus HKU1* and *OC43*) [Kuypers et al., 2007; Dominguez et al., 2009; Finkbeiner et al., 2009a,b]. Each pool containing RNA from six samples was run on each protocol. If a pool was positive for a given assay, RNA from each of the six samples was run individually to determine which of the samples were positive. Negative and positive controls were included in each run for all protocols. Due to concern for PCR inhibitors in stool, samples were spiked with $10\mu\text{L}$ of extracted RNA from each pool with a known quantity of pandemic 2009 H1N1 influenza A RNA, and then assessed for influenza RNA by RT-PCR (Supplemental Table).

Norovirus-positive samples were randomly selected for sequencing during the peak of activity, and conventional PCR targeting a 343-nucleotide region of the capsid gene was performed using a previously published assay [Mans et al., 2010; De Benedictis et al., 2011]. Amplicons were analyzed by agarose gel electrophoresis and sequenced on an ABI 3730 DNA sequencer (Applied Biosystems Technologies, Carlsbad, CA) at the University of Colorado School of Medicine Cancer Center DNA Sequencing and Analysis Core.

Data Analysis

Specimens were decoded, and medical chart review was performed on virus-positive subjects using a standardized form. In order to assess typical clinical features of the viruses, clinical data comparisons were limited to patients who were not immunocompromised and who were positive for only one virus.

Study data were collected and managed using RED-Cap electronic data capture tools hosted at the UCSOM [Harris et al., 2009; Chhabra et al., 2013]. Statistical computations were conducted with SAS software, version 9.1.3. Significance was determined using the Wilcoxon Signed Rank test or Chi Square.

RESULTS

Patients

From September 2006 to June 2009, 1777 stool samples were submitted for viral diagnostic testing. Of these, 1105 (62%) had enough residual sample to permit inclusion in the study. There were no differences in the age, gender, or order location of patients with samples included in or excluded from the study. The majority (941, 85%) of samples were from pediatric patients (median age = 2.97 years, 54% male) (Table I). The remaining 164 samples were referral specimens submitted from adults seen at the University of Colorado Hospital (UCH) from September 2006 to December 2008, of which 141 (86%) were submitted between September 2006 and January 2007.

Virus Detection

All pools were positive for influenza, indicating the absence of PCR inhibitors. Viral RNA/DNA was detected in 247 (26.2%) of the 941 pediatric samples. Of these, 95 (10.0%) were positive for adenovirus, 33 (3.5%) for astrovirus, 8 (0.9%) for norovirus GI, 90 (9.6%) for norovirus GII, 49 (5.2%) for rotavirus, and 2 (0.2%) for sapovirus. Coronavirus RNA was not detected in any sample (Table II). Overall, 100 (27.3%) outpatient samples, 80 (32.4%) inpatient samples, and 67 (27.1%) samples from immunocompromised patients were positive for at least one virus (Table II). Evidence of co-infection was present in 28 (3.0%) of pediatric samples (Table III).

Each virus demonstrated seasonal and variable changes from year to year (Fig. 1). Adenovirus was detected throughout the year in children with acute gastroenteritis, with highest prevalence (44.5%) in April 2009. Astrovirus was detected from January to

TABLE II. Incidence of Viral Gastroenteritis in Pediatric and Adult Patients

	Total (n = 1105)	Pediatric (n = 941)	Adult (n = 164)
<i>Overall</i>	311 (28.1)	247 (26.2)	65 (39.6)
<i>Adenovirus</i>	99 (9.0)	95 (10.0)	4 (2.4)
<i>Astrovirus</i>	35 (3.2)	33 (3.5)	2 (1.2)
<i>Coronavirus</i>	0 (0.0)	0 (0.0)	0 (0.0)
<i>Norovirus GI</i>	14 (1.3)	8 (0.9)	6 (3.7)
<i>Norovirus GII</i>	141 (12.8)	90 (9.6)	51 (31.1)
<i>Rotavirus</i>	50 (4.5)	49 (5.2)	1 (0.6)
<i>Sapovirus</i>	2 (0.2)	2 (0.2)	0 (0.0)

August 2007, with highest prevalence (15%) in April 2007; however, in 2008 and 2009 was only detected January to March. Norovirus GII was most prevalent from October 2006 to April 2007, and then was not detected until April 2008 with an increase in prevalence in December 2008. Rotavirus was highly prevalent from October 2006 to April 2007 with decreasing prevalence thereafter.

Of the 164 adult samples tested, 64 (39%) were positive, of which 51 (80%) contained norovirus GII nucleic acid, 4 (2.4%) adenovirus, 2 (1.2%) astrovirus, 6 (3.6%) norovirus GI, and 1 (0.6%) rotavirus as shown in Table II. All 51 samples positive for norovirus GII were collected between October 2006 and January 2007, and 8 of 14 (57%) samples were positive in October, 11 of 27 (41%) in November, 22 of 58 (38%) in December, and 10 of 41 (24%) in January. During this timeframe, 12 adult and 7 pediatric, norovirus positive samples were sequenced: 16 (84%) were norovirus GII.4. and 3 (16%), all from pediatric patients, were norovirus GII.14.

Persistent Infection

329 samples were collected from 112 patients. Of these, 18 patients had multiple samples positive for at least one virus. Five immunocompromised patients in this study had multiple, serial stool samples positive for the same virus. Details of these clinical cases are outlined in Table V.

TABLE I. Characteristics and Patient Location on Submission of Stool Samples

	Total (n = 247)	Adenovirus (n = 95)	Astrovirus (n = 33)	Norovirus GI (n = 8)	Norovirus GII (n = 90)	Rotavirus (n = 49)	Co-infections (n = 28)
Demographics							
Male	122 (49.3)	48 (50.5)	18 (54.5)	5 (62.5)	46 (51.1)	25 (51.0)	18
Median age (years)	2.97	2.61	1.99	1.97	2.56	1.13	1.14
Outpatient (n = 366)	100 (40.4)	42 (44.2)	14 (42.4)	3 (37.5)	36 (40.0)	22 (44.9)	10 (35.7)
Clinic (n = 238)	69 (27.9)	26 (27.3)	11 (33.3)	0 (0.0)	22 (24.4)	17 (34.7)	8 (28.6)
ED/Urgent Care (n = 138)	31 (12.6)	16 (16.8)	3 (9.0)	3 (37.5)	14 (15.6)	5 (10.2)	2 (7.1)
Inpatient (n = 364)	80 (32.4)	34 (35.8)	9 (27.3)	2 (25.0)	26 (28.9)	18 (36.7)	10 (35.7)
Medical Floor (n = 303)	68 (27.5)	29 (30.5)	8 (24.2)	2 (25.0)	22 (24.4)	15 (30.6)	9 (32.1)
ICU (n = 61)	12 (4.8)	5 (5.3)	1 (3.0)	0 (0.0)	4 (4.4)	3 (6.1)	1 (3.5)
Immunocompromised (n = 211)	67 (27.1)	25 (26.3)	10 (30.3)	3 (37.5)	28 (31.1)	9 (18.4)	8 (28.6)

TABLE III. Mixed Infections Identified in 28 Patients With Gastrointestinal Symptoms

Viruses	No. of samples
<i>Adenovirus, astrovirus</i>	1
<i>Adenovirus, norovirus GI</i>	1
<i>Adenovirus, norovirus GII</i>	6
<i>Adenovirus, rotavirus</i>	14
<i>Astrovirus, norovirus GII</i>	1
<i>Norovirus GII, rotavirus</i>	2
<i>Adenovirus, rotavirus, norovirus GII</i>	2
<i>Norovirus GI, norovirus GII, rotavirus</i>	2

Clinical Data

The demographic and clinical characteristics of the 135 non-immunocompromised patients with singly positive samples and clinical data available are shown in Table IV. The median duration of diarrhea was 6 days (IQR 3-14), with a median of 6 stools per day (IQR 4-10) per patient. In total, 56% of patients had emesis and 41.6% had fever. Approximately one-quarter of the patients had fecal leukocytes and erythrocytes present. Patients with adenovirus were more likely to have decreased urine output (28.6%). There were no other statistically significant differences in symptoms or laboratory values among the different enteric viruses.

Performance Characteristics: RT-PCR versus EM

Samples positive by RT-PCR for adenovirus, rotavirus, and caliciviruses or astroviruses were compared

with those positive by EM (Table VI). Of 111 samples positive for adenovirus by EM or PCR, 99 (89%) were positive by PCR and 12 (11%) were positive by EM. Of 192 samples positive for astrovirus, sapovirus, or norovirus by EM (designated collectively as “small round viruses”) or RT-PCR, 186 (97%) were positive by RT-PCR and 21 (11%) were positive by EM. For rotavirus, 50 (60%) were positive by RT-PCR and 56 (67%) were positive by EM.

DISCUSSION

This is the first hospital-based, 3-year, longitudinal study evaluating multiple viral etiologies of acute gastroenteritis in pediatric patients in the United States. Overall, 26% of all samples from children with gastrointestinal symptoms showed evidence of viral infection by RT-PCR, similar to what has been reported in most studies using molecular techniques [Esper et al., 2010; Chhabra et al., 2013; Jevšnik et al., 2013; Payne et al., 2013]. Of note, viral agents were not detected in 74% of samples, and few samples had bacterial agents identified, suggesting that diarrhea had a non-infectious cause or was due to a pathogen not assessed in this study. Recently, Payne et al. prospectively evaluated the etiology of acute gastroenteritis in children less than 5 years of age for 12 months in three counties in the United States and found that 21% of cases were attributed to norovirus and 12% to rotavirus. When Chhabra et al. expanded this study to evaluate the roles of bocavirus, parechovirus, and aichivirus in acute

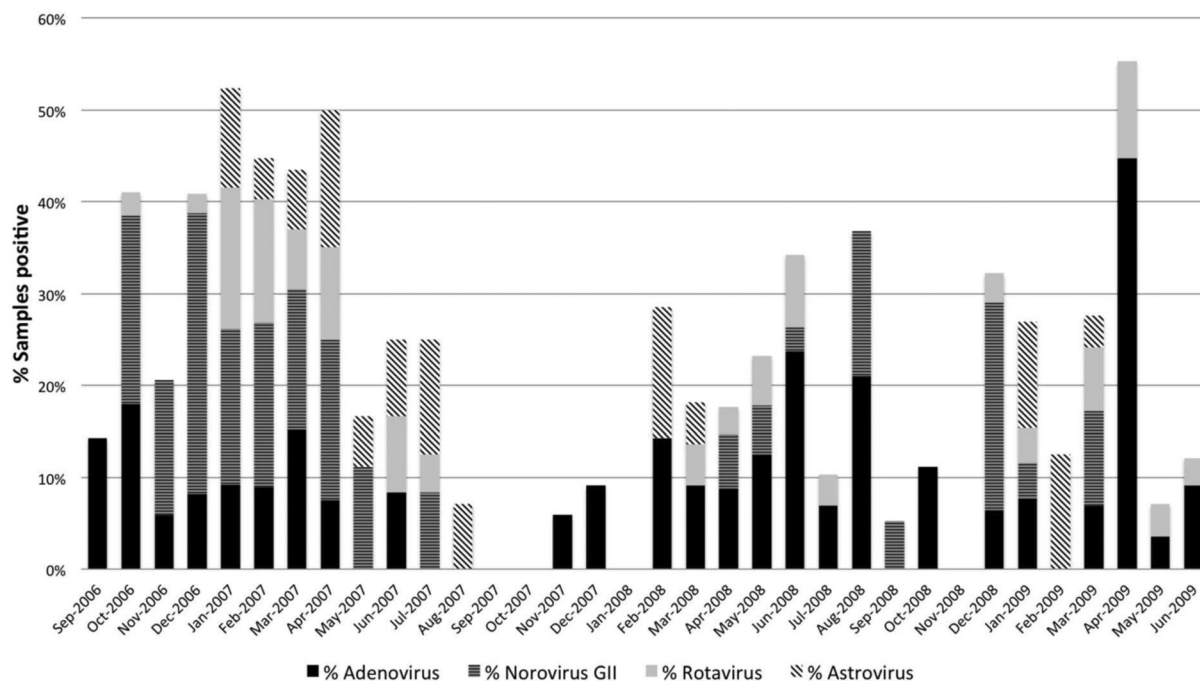


Fig. 1. Distribution of samples from pediatric patients positive for any virus submitted for viral diagnostic testing at Children's Hospital Colorado from September 2006 through June 2009.

TABLE IV. Clinical Characteristics, Laboratory Findings, and Patient Management of Non-Immunocompromised Patients Where Data was Available

	Total (n = 135)	Adenovirus (n = 42)	Astrovirus (n = 10)	Norovirus GII (n = 57)	Rotavirus (n = 26)	P-value*
Demographics						
Sex (male)	64 (51.2)	23 (54.8)	4 (40.0)	3 (53.1)	10 (38.5)	0.34
Age (years) ^a	1.46 (0.58–5.2)	1.24 (0.51–4.9)	0.97 (0.08–5.0)	1.74 (0.58–5.2)	1.04 (0.61–6.8)	0.91
Underlying medical condition	56 (44.8)	17 (40.5)	3 (30.0)	29 (50.9)	10 (38.5)	0.45
Known sick contacts	22 (17.6)	8 (19.0)	2 (20.0)	11 (19.3)	3 (11.5)	0.66
History and Physical Exam						
Duration of symptoms (days) ^a	6 (3–13)	7 (4–20)	6 (4–17)	5 (3–16)	6 (3–11)	0.77
Diarrhea	121 (89.6)	40 (95.2)	8 (80.0)	49 (86.0)	24 (92.3)	0.28
Blood visible in stool	36 (26.7)	13 (31.0)	3 (30.0)	15 (26.3)	5 (19.2)	0.56
Mucous visible in stool	19 (15.2)	6 (14.3)	1 (10.0)	10 (17.5)	3 (11.5)	0.76
Duration of diarrhea (days) ^a	6 (3–14)	6 (0.5–12)	9 (1.5–49)	5 (3–16)	7 (4–13)	0.30
Max no. of stools per day ^a	6 (4–10)	6 (4–12)	5 (3–7)	6 (4–10)	7 (4–10)	0.91
Emesis	70 (56.0)	24 (57.1)	7 (70.0)	30 (52.6)	16 (61.5)	0.74
Duration of emesis (days) ^a	4 (2–6)	4 (2.5–7)	4 (3–6)	2 (2–5)	6 (4–8)	0.10
Max no. of emesis per day ^a	3 (2–5)	3 (2–4)	5 (1–6)	3 (2–5)	4 (3–6)	0.52
Fever (> 38.3 °C)	52 (41.6)	18 (42.9)	5 (50.0)	24 (42.1)	10 (38.5)	0.93
Abdominal cramping	15 (12.0)	4 (9.52)	2 (20.0)	8 (14.0)	3 (11.5)	0.79
Abdominal distention	23 (18.4)	7 (16.7)	0 (0.0)	13 (22.8)	3 (11.5)	0.44
Abdominal tenderness	20 (16.0)	7 (16.7)	1 (10.0)	12 (21.0)	1 (3.9)	0.14
Lethargic	41 (32.8)	12 (28.6)	2 (20.0)	21 (36.8)	8 (30.8)	0.67
Decreased oral intake prior to testing	65 (52.0)	22 (52.4)	6 (60.0)	29 (50.9)	14 (53.9)	0.97
Decreased urine output	21 (16.8)	12 (28.6)	1 (10.0)	7 (12.3)	2 (7.7)	0.038
Hypotension (out of normal range for age)	8 (6.4)	3 (7.1)	1 (10.0)	3 (5.3)	2 (7.7)	0.89
Tachycardia	37 (29.6)	14 (33.3)	1 (10.0)	18 (28.1)	11 (24.4)	0.80
Stool studies						
Red blood cells present	29 (23.2)	11 (26.2)	2 (20.0)	14 (24.6)	4 (15.4)	0.56
White blood cells present	32 (25.6)	12 (28.6)	1 (10.0)	17 (29.8)	3 (11.5)	0.18
Bacterial culture positive	7 (5.6)	3 (7.1)	0 (0.0)	4 (7.0)	0 (0.0)	0.38
Positive for <i>C. difficile</i>	6 (4.8)	0 (0.0)	0 (0.0)	4 (7.0)	2 (7.7)	0.20
Management						
Received IV fluids	32 (25.6)	11 (26.2)	3 (30.0)	13 (22.8)	8 (31.0)	0.74
Admitted to hospital	79 (63.2)	27 (64.3)	6 (60.0)	34 (59.7)	18 (69.2)	0.85
Admitted for GI reasons	60 (48.0)	21 (50.0)	6 (60.0)	26 (45.6)	13 (50.0)	0.89
Admitted to pediatric ICU	17 (13.6)	4 (9.5)	1 (10.0)	9 (15.8)	4 (15.4)	0.64
Length of stay (days) ^a	8 (4–15)	7 (4–20)	4 (3–6)	9 (5–15)	11 (4–23)	0.48

^aindicates median number and interquartile range.

*statistical analysis performed on adenovirus, rotavirus, and norovirus GII samples as clinical data was available on sufficient number of patients.

gastroenteritis in addition to the viruses studied here, viruses were identified in two-thirds of their patients. This increased identification of an etiological agent is likely due to their active surveillance and more comprehensive survey. Additionally, there are reports of diarrhea associated with rotavirus groups B in children in Asia [Sanekata et al., 2003] and group C worldwide [Jiang et al., 1995; Kumazaki and Usuku, 2013], as well as toroviruses [Koopmans et al., 1997; Jamieson et al., 1998] in children with gastroenteritis, which were not included in the RT-PCR assay in this study. Nevertheless, these studies suggest that there are likely other, yet to be discovered, viruses that cause acute gastroenteritis in children.

As expected, the incidence of rotaviruses decreased over the course of this study, which corresponded with the implementation of the rotavirus vaccine, RotaTeq (Merck, USA) in 2006 and Rotarix (GlaxoSmithKline, Belgium) in 2008. Interestingly, norovirus

GII was detected more frequently than rotavirus both prior to and following vaccine implementation, which indicates a significant, continuous burden of disease due to norovirus infections. This correlates with recently published data [Payne et al., 1986, 2013; Risku et al., 2010; Sánchez-Fauquier et al., 2011]. A significant amount of adenovirus DNA was detected in children with gastrointestinal symptoms, and while other studies have shown variable detection rates of adenovirus 40 and 41 [Colomba et al., 2006; Chhabra et al., 2013] the assay in this study was able to detect multiple serotypes in addition to 40 and 41. Only serotypes 40 and 41, and occasionally 18 and 31 have been associated with gastroenteritis. Despite the fact that providers at this institution are discouraged from sending samples for EM unless the patient is symptomatic, the numbers in this study may overestimate the burden of gastroenteritis in children attributed to adenoviruses and could represent asymptomatic

TABLE V. Duration of Viral Shedding in Consecutive Stool Samples in Five Immunocompromised Hosts

Patient	Age/Sex	Underlying medical condition	Related treatment & complications	Virus	No. Samples	No. Weeks	Clinical Course
1	11 years, female	Beta thalassemia	URCB-HSCT, GVHD	Norovirus GII	6	25	Diarrhea, cramping abdominal pain, emesis January – April 2007, new onset diarrhea, abdominal pain in July 2007
2	2 years, male	Severe infantile osteoporosis	URB-HCST, GVHD	Norovirus GII	4	32	Symptomatic gastroenteritis October 2006 – May 2007 with failure to thrive
3	5 months, female	Severe combined immunodeficiency	Bone marrow transplants (2)	Norovirus GII	2	5	Hospitalized May 2008 – August 2009 with persistent diarrhea, intermittent adenovirus, rotavirus, and norovirus GII positive samples; 2 consecutive norovirus GII samples
4	6 years, male	Severe aplastic anemia	URCB-HCST	Norovirus GII	2	18	Diarrhea, emesis March 2007; recurrent diarrhea in July 2007
5	20 months, male	Atypical rhabdoid tumor	Chemotherapy, multiple ASCTs	Astrovirus	4	12	Chemotherapy associated diarrhea and emesis, requiring multiple admissions, one episode associated with hypovolemic shock

URCB-HSCT, unrelated cord blood hematopoietic stem cell transplant; GVHD, graft-versus-host disease; AGE: acute gastroenteritis; ASCT, autologous stem cell transplant.

shedding of non-enteric adenoviruses with other pathogens responsible for symptoms.

Astroviruses have long been known to cause gastroenteritis in humans [De Benedictis et al., 2011; Chhabra et al., 2013]. Recently, however, new astrovirus strains have been discovered and been associated with disease in humans [Zimmerman et al., 2001; Finkbeiner et al., 2008, 2009a,b; Hall, 2011; Lindesmith et al., 2011]. The astrovirus protocol used in this study was not designed to detect these novel strains and likely underestimates the true burden of astrovirus disease in these study patients [Phan et al., 2007; De Benedictis et al., 2011]. Surprisingly, in contrast to other studies, very few cases of

sapovirus were detected. In other studies, sapoviruses have been reported to cause more vomiting than diarrhea in children, and this study only evaluated stool samples [Chhabra et al., 2013]. Additionally, no cases were associated with the known endemic human coronaviruses. While similar results have been documented in the literature [Esper et al., 2010; Jevšnik et al., 2013], other studies have found endemic coronaviruses and coronavirus-like particles in stool in patients with gastroenteritis [Payne et al., 1986; Risku et al., 2010]. The data from this study supports the hypothesis that known endemic coronaviruses do not cause clinically significant gastroenteritis in children.

All viruses demonstrated seasonal as well as annual variation throughout the study period. Adenovirus was present throughout the year, while astrovirus, rotavirus, and norovirus GII were present most frequently in the fall and winter months, similar to previously reported data [Zimmerman et al., 2001; Hall, 2011; Chhabra et al., 2013]. The data in this study indicate that there was an outbreak of norovirus GII.4 in this community during the winter of 2006–2007. This observation supports other data which suggests norovirus GII.4 strain is the leading cause of norovirus outbreaks due to its ability to rapidly evolve through antigenic variation, possibly driven by herd immunity [Lindesmith et al., 2011] as well as recombination [Bull et al., 2007; Phan et al., 2007; Unicef WHO, 2009]. These data also support the observation that multiple different strains of

TABLE VI. Performance Characteristics: RT-PCR versus Electron Microscopy for Detection of Virus in Stool Samples

Virus	EM +	EM –	Total
Adenovirus			
PCR +	12	87	99
PCR –	12	994	1006
Total	24	1081	1105
Rotavirus			
PCR +	22	28	50
PCR –	34	1021	1055
Total	56	1049	1105
SRV/Norovirus/Astrovirus			
PCR +	15	177	192
PCR –	6	907	913
Total	21	1084	1105

norovirus can circulate in the community at one time, which could facilitate evolution of new strains.

It is unclear how long virus shedding can occur in the intestinal tract following natural infection. Several publications have shown variable duration of shedding in pediatric patients both with and without symptoms [Payne et al., 1986, 2013; Wilde et al., 1991; Mitchell et al., 1995; Richardson et al., 1998; Parashar et al., 2003; Wilhelmi et al., 2003; Colomba et al., 2006; Unicef WHO, 2009; Graham et al., 2010; Churgay and Aftab, 2012; Chhabra et al., 2013; Jevšnik et al., 2013]. Data from this study indicate that immunocompromised children, in particular those who have received HSCTs, may be infected and shed enteric viruses for up to 8 months and are at risk for persistent symptomatic infection. These findings have significant clinical and infection control implications and complicate the diagnosis of diarrhea in these study patients. Immunocompromised patients might also provide a reservoir for these viruses and a potential environment for recombination events and generation of novel viral strains [Booth et al., 2003; Choi et al., 2003; Hsu et al., 2003; Peiris et al., 2003; Hsueh et al., 2004; Schorn et al., 2010]. Furthermore, prolonged infection with enteric pathogens has been shown to put patients at risk for significant sequelae including severe weight loss, malnutrition, dehydration, and altered intestinal mucosa [Roddie et al., 2009; Schwartz et al., 2011; Jevšnik et al., 2013].

This study did not demonstrate statistically significant differences in history, physical examination, laboratory studies, or patient management among illnesses caused by different enteric viruses. However, while enteric viruses are generally thought not to elicit severe inflammatory gastroenteritis, nearly one quarter of patients with viral acute gastroenteritis in this study had erythrocytes and leukocytes present in their stool. Additionally, a significant number of pediatric patients with viral acute gastroenteritis were hospitalized due to gastrointestinal symptoms, and a small number were admitted to the intensive care unit, which indicates these infections can have significant financial impacts.

In the past decade, many clinical laboratories have been moving toward RT-PCR multiplex assays to assess the etiology of multiple infectious diseases. At the time these samples were collected and submitted for diagnostic testing, this institution only employed EM for viral testing of stool samples. These results clearly demonstrate that RT-PCR is superior in the detection of noroviruses, astroviruses, and adenoviruses in stool than EM. RT-PCR did not perform significantly better than EM in the diagnosis of rotavirus, but this may have been due to the fact that the RT-PCR assay in use detected only group A rotaviruses, while EM was able to detect groups A-C. Additionally, the assays used in this study were designed for laboratory research and have not been fully vetted for clinical samples.

This study has several limitations. It was retrospective and conducted at a large tertiary care children's hospital, which likely biased sample collection toward more seriously ill patients and those with underlying medical conditions and therefore may not reflect the burden of disease in the wider pediatric population. Providers at the institution in this study are discouraged from routinely sending stool samples in patients with acute gastroenteritis unless it will impact clinical care, and this may have caused selection bias toward children with severe symptoms. Diluted stool samples were used, which might have reduced the ability to detect viral nucleic acid present in small amounts. Additionally, the presence of viral nucleic acid in stool may not reflect the cause of current symptoms but could instead reflect prolonged shedding from a previous infection or asymptomatic carriage [Kapusinszky et al., 2012].

In summary, a viral cause was identified in 26% of children with diarrheal disease, with the largest burdens of disease attributed to adenoviruses and noroviruses in children. This study indicates that immunocompromised patients can experience prolonged symptomatic infection and shedding of enteric virus. The data in this study also support the use of improved rapid, well-standardized RT-PCR diagnostic methods to establish the etiology of diarrhea. In addition to the advances made in the prevention of rotavirus disease, development of targeted therapies and vaccines towards other enteric viruses are needed to alleviate the significant burden of disease associated with diarrheal illness in children.

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