Noroviruses: The Most Common Pediatric Viral Enteric Pathogen at a Large University Hospital After Introduction of Rotavirus Vaccination

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We conducted an 8.5-year study examining enteric viruses at Texas Children's Hospital (TCH) before and after rotavirus vaccine introduction. Norovirus prevalence was 10.9%. Rotavirus prevalence decreased 64% after vaccine licensure. Noroviruses are the most common TCH enteropathogen and will likely eclipse rotaviruses as the most important US pediatric gastroenteritis pathogen.

Key words. Norovirus; Rotavirus; Gastroenteritis; Children; Vaccine

(See the Editorial Commentary by Yen and Hall on pages 61–2.)

Rotaviruses (RVs) and noroviruses (NoVs) are the 2 most important acute gastroenteritis enteropathogens in children. NoVs are the leading cause of gastroenteritis in the US. Genogroup II, genotype 4 (GII.4) NoV strains are the predominant circulating genotype [1]. RVs are the most common cause of severe gastroenteritis in children <5 years of age worldwide [2, 3], but NoV gastroenteritis in children is increasingly being recognized [4].

In 2006, the Advisory Committee on Immunization Practices recommended for all US infants to receive rotavirus vaccination [5]. Despite relatively low US vaccination rates [6], a 60%–87% reduction in rotavirus gastroenteritis has been noted since vaccine licensure [6, 7]. We conducted a longitudinal epidemiologic study to compare norovirus and rotavirus prevalence in pediatric stools submitted to a large university hospital before and after rotavirus vaccine introduction.

METHODS

Study Population and Sample Collection

Stools submitted to Texas Children's Hospital (TCH) in Houston, Texas for viral enteropathogen testing from February 2002 to June 2010 were examined. For this epidemiologic surveillance study, only age and gender demographics were retrieved. No data were available from May to July 2006. The Baylor College of Medicine Institutional Review Board approved this study.

Rotavirus and Adenovirus Detection

Rotaviruses and adenoviruses were detected by electron microscopy (EM). From 2002 to 2008, when RV frequency increased from January to April, a membrane-based immunogold assay was used as the initial assay, and if negative, the sample was tested by EM. From 2009, this RV assay was used routinely with secondary EM analysis on rotavirus-negative samples from January 1st to June 1st. Remaining stools were stored at –70°C for norovirus testing.

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Norovirus Detection

Ten percent stool suspensions in MilliQ water were diluted 1:100 and 1:50 for conventional (cRT-PCR) or real-time reverse-transcription polymerase chain reactions (rRT-PCR), respectively. RNA was extracted by heat release [8].

From February 2002 to March 2004, all stools were screened for NoVs by cRT-PCR with region B primers (Table 1 in the Supplementary Appendix). After March 2004, only stools >2 grams were tested with these primers. For GI NoV cRT-PCR confirmation, G1SKR and G1SKF primers (Supplementary Table 1) were used, with 52°C PCR annealing temperature. All cRT-PCR-positive samples were confirmed by Southern hybridization, using Mon 458 and Mon 459 probes (Supplementary Table 1) [8]. SRI-3 or NVp160 GI probe was also used.

Due to time and labor constraints, rRT-PCR was performed for a convenience sample of ∼10% of 2006–2010 stools distributed throughout each year and selected in a systematic fashion without prior clinical or laboratory information. A 30 µL mixture of 10 µL RNA, 15 µL TaqMan Universal PCR Master Mix, and 0.75 µL 40× MultiScribe and RNase Inhibitor (Applied Biosystems), 150 nM of NV1LCR, QNIF4 GI primers or COG2R, QNIF2d GII primers, and 200 nM GI NVGGI or GII QNIFS probe was prepared (Supplementary Table 1). RT-PCR amplification was performed with a StepOne ABI platform (Applied Biosystems): 48°C for 30 minutes; 95°C for 10 minutes; then 45 cycles of 95°C for 15 seconds and 60°C for 1 minute.

A subset of norovirus amplicons were sequenced (Genewiz or Seqwright) after PCR Clean-up Kit purification (QIAGEN). Larger genome sequences were determined for a few strains (GenBank accession numbers AB365435, EU310927, JF827296, JN005886).

Statistical Analysis

Prevaccination stool specimens (2002–2006) were compared with postvaccination stools (2007–2010). Significant proportional differences were evaluated with χ^2 analysis; medians were compared by Wilcoxon rank-sum test using STATA (version 9.2) software.

RESULTS

Stool specimens from 8173 subjects were collected over 8.5 years (Table). The median age of enrolled children was 21 months. The annual median age steadily increased from 14.2 to 47 months over the study period, with the overall median age increasing significantly from 18.8 months during 2002–2006 to 25.4 months for 2007–2010 ($P < .01$). Three thousand two hundred twenty-two (39.4%) stools were evaluated for NoVs, with an overall prevalence of 10.9%. NoV detection ranged from 5.6% to 16.8% between 2002 and 2006 and from 12.1% to 14.9% between 2007 and 2010. GII NoVs predominated, with the GII.4 NoV genotype most commonly identified each year (Supplementary Table 2). NoV prevalence increased from 10.1% to 180.3% for all pediatric age groups <60 months over the 2 time periods, with the greatest increase in children aged 12–17 months (Supplementary Table 3). Overall NoV-positive pediatric median age was 18 months; median age increased from 17.2 to 21.2 months from 2002–2006 to 2007–2010, respectively.

NoVs were detected in 52 stools by rRT-PCR from 2006 to 2010. One stool was positive for both GI and GII NoVs. Median cycle threshold value for NoV detection was 27. Both conventional and real-time RT-PCR assays were performed for 78 stools with 95% concordance. Among 4 discordant samples, 3 were positive only by cRT-PCR, whereas one was positive only by rRT-PCR.

The mean annual prevalence of RV-positive stools was 14.6% from 2002 to 2006 compared with 5.2% from 2007 to 2010, representing a 64% decrease after RV vaccine introduction ($P < .001$). RV prevalence ranged from 8.5% to 18.7% before vaccine introduction but declined steadily from 9.2% to 2.7% in the postvaccination period (Table), despite similar annual medians of tested stools between 2002 and 2006 (n = 1007) and between 2007 and 2009 (n = 1030). Seasonal RV peaks alternated each year between February and April in both prevaccine and postvaccine eras (Supplementary Figure). However, the median seasonal peak percentage of RV-positive stools declined significantly from 35.2% during 2002–2006 to 15.9% for 2007–2010, with a 55% decrease between the 2 periods ($P < .01$).

Overall median age for RV-positive children was 14 months; median age increased from 13.7 to 17.6 months from 2002–2006 to 2007–2010, respectively. Children most commonly RV-infected were 12-17 months of age from 2002 to 2006 but aged 18–23 months from 2007 to 2010 (Supplementary Table 3). The greatest reduction in RV activity after vaccine introduction was observed in children <36 months, with 51.2%–77.6% decreases. This age group represents children most likely to have received RV vaccination.

Table. Prevalence of Viral Enteric Pathogens in Texas Children's Hospital Stool Specimens

Year	No. of RV- and Adenovirus-Tested Samples	Median Age of Tested Children (Months)	Rotavirus Positive (%)	Adenovirus Positive (%)	No. of NoV-Tested Samples	NoV Positive (%)	GI NoV Positive (%)	GII NoV Positive (%)
2002	638	14.2	119 (18.7)	10 (1.6)	637	49 (7.7)	0	49 (100)
2003	786	18.5	67 (8.5)	14 (1.8)	785	44 (5.6)	10 (22.7)	34 (77.3)
2004	1120	19.0	153 (13.7)	29 (2.6)	631	106 (16.8)	1 (0.9)	105 (99.1)
2005	1100	19.4	157 (14.3)	10 (0.9)	224	34 (15.2)	0	34 (100)
2006	1007	19.8	183 (18.2)	10 (1.0)	283	31 (11.0)	0	31 (100)
2007	1095	20.7	101 (9.2)	13 (1.2)	215	32 (14.9)	3 (9.4)	29 (90.6)
2008	1030	22.4	50 (4.9)	21 (2.0)	207	25 (12.1)	1 (4.0)	24 (96)
2009	947	29.3	40 (4.2)	16 (1.7)	163	20 (12.3)	1 (4.2)[a]	20 (100)[a]
2010	450	47.0	12 (2.7)	7 (1.6)	77	11 (14.3)	0	11 (100)

Abbreviations: GI, genogroup I; GII, genogroup II; NoV, norovirus; RV, rotavirus.

[a]One sample tested positive for both GI and GII NoVs.

However, RV-positive stools also decreased 6%–55.6% in older children unlikely to have received RV vaccination.

Adenovirus detection was uncommon, ranging from 0.9% to 2.6% between 2002 and 2006 and from 1.2% to 2.0% between 2007 and 2010. RVs were identified in 15 NoV-positive stools; adenoviruses were identified in 6 NoV-positive stools.

DISCUSSION

Rotaviruses and NoVs are the 2 most important causes of pediatric gastroenteritis in industrialized countries [2, 4]. With declining rotavirus activity, NoVs have emerged as the most common pediatric viral enteropathogen at TCH.

One of this study's strengths is the large number of stool samples examined over 8.5 years. Overall norovirus prevalence was 10.9%, with detection increasing after 2003, consistently ranging between 11% and 16.8% of stools. A similar increase in NoV incidence was observed in the United States and other developed nations from 2002 to 2004, which was attributed to emergence of GII.4 NoV variants [9]. GII NoV predominance at TCH is consistent with other NoV non-outbreak reports [4, 10]. As rotavirus vaccination coverage increases, NoVs will soon eclipse RVs as the most important cause of US pediatric gastroenteritis.

Our 8.5 year surveillance supports the national pattern of decreasing rotavirus activity since RV vaccine introduction. There was a 64% decrease in overall rotavirus prevalence and a 55% decrease in median seasonal peak percentage after 2006. The median age of children who submitted stools (18.8 to 25.4 months), with RV-positive stools (13.7 to 17.6 months), and with NoV-positive stools (17.2 to 21.2 months) increased between the 2 time periods. Although these changes may reflect a shift in viral gastroenteritis epidemiology, with NoV predominance in older children, NoV and rotavirus detection were most common in children <3 years of age before and after RV vaccine licensure. Changing patterns of physician utilization of viral diagnostics may also have contributed to the rise in pediatric age. RV detection decrease (up to 55.6%) in children too old to have received the vaccine (ie, $\geq$60 months of age) suggests herd immunity.

Study limitations include evaluation of a single institution, inclusion criteria of only a physician request for fecal virologic testing, and lack of a healthy control population in whom the prevalence of asymptomatic infection with these viral enteropathogens could be determined. Two case-control studies at our institution during the 2008 and 2009 RV peak seasons reported higher rotavirus prevalences (44% and 21%, respectively) in much more selected populations of <2 years (n = 205) and <3 years (n = 181) of age, respectively, with strictly defined gastroenteritis ($\geq$ 3 loose stools or $\geq$ 1 vomiting episodes within 24-hours) [11, 12]. Peak monthly RV detection was 18.3% and 13.5% in our study during these RV peak seasons, respectively. These RV frequency differences likely reflect patient screening and selection differences; the current study evaluated all stools tested for viral enteropathogens. We were also unable to assess causal relationship between viral detection and the patient's clinical syndrome because limited clinical information was

collected. Finally, different NoV methods were used in the study, with convenience sampling of stools selected for rRT-PCR. However, high concordance (95%) between the RT-PCR assays lends confidence that NoV frequency was not affected.

Noroviruses have emerged as the predominant pediatric viral enteric pathogen at TCH after rotavirus vaccine introduction. Norovirus prevalence remained stable or increased, whereas rotavirus activity dramatically decreased. As rotavirus vaccine coverage increases, rotavirus and acute pediatric gastroenteritis prevalence will likely continue to decline locally and across the nation. Recognition of NoVs as the most important cause of acute pediatric gastroenteritis is imminent in the United States and possibly in other regions of the world where rotavirus vaccination is implemented successfully. Our findings underscore the urgent need for the development of an effective norovirus vaccine for children.

Supplementary Data

Supplementary materials are available at the *Journal of The Pediatric Infectious Diseases Society* online (http:/jpids.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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