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Norovirus Activity and Genotypes in Sporadic Acute Diarrhea in Children in Shanghai During 2014–2018

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Background: Based on the impact public health of norovirus and the current progress in norovirus vaccine development, it is necessary to continuously monitor the epidemiology of norovirus infection, especially in children who are more susceptible to norovirus.

Objectives: To monitor the activity and genotypes of norovirus infection in sporadic diarrhea in Shanghainese children during 2014–2018.

Study design: Acute diarrheal cases were prospectively enrolled in the outpatient setting. Real-time reverse transcription-polymerase chain reaction was used for screening norovirus GI and GII genogroups. Dual norovirus genotypes were identified based on the partial capsid and polymerase gene sequences.

Results: Of the 3422 children with diarrhea, 510 (14.9%) were positive for noroviruses with 13 (2.5%) strains being GI genogroup and 497 (97.5%) strains being GII genogroup. Five distinct capsid GII genotypes were identified, including GII.4-Sydney/2012 (71.8%), GII.3 (13.8%), GII.17 (7.8%), GII.2 (6.0%), GII.6 (0.3%) and GII.8 (0.3%). Seven polymerase GII genotypes were identified, including GII.Pe (77.0%), GII.P12 (11.0%), GII.P17 (9.0%), GII.P16 (2.1%), and GII.P7, GII.P8 and GII.P2 in each (0.3%). Eleven distinct polymerase/capsid genotypes were identified with GII.Pe/GII.4-Sydney/2012 (74.2%), GII.P12/GII.3 (11.7%) and GII.P17/GII.17 (7.7%) being common. GII.P17/GII.17 strains were detected since September 2014. Recombinant GII.P16/GII.2 strains were detected since December 2016.

Conclusions: Norovirus is a major pathogen causing diarrhea in Shanghainese children. GII.Pe/GII.4-Sydney/2012 strains remained the predominant genotype. The emergence of GII.P17/GII.17 and GII.P16/GII.2 strains in sporadic diarrhea was consistent with norovirus-associated outbreaks attributable to these 2 novel variants in China. Continuous monitoring norovirus genotypes circulating in pediatric population is needed for current vaccine development.

Key Words: norovirus, genotypes, diarrhea, children

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Human noroviruses are the leading cause of epidemic and sporadic acute gastroenteritis in people of all ages worldwide and are associated with 18% of all acute gastroenteritis and more than 212,000 deaths annually.[1,2] Globally, noroviruses are also the leading cause of foodborne illness and estimated to be responsible for 125 million foodborne illnesses annually.[3]

Noroviruses belong to the family Caliciviridae and are genetically classified into at least 7 genogroups (G), of which GI, GII and GIV infect humans.[4] GII and GI are responsible for the majority of human disease. A dual-nomenclature system has been proposed for GI and GII noroviruses based on viral capsid protein (VP1) and RNA-dependent, RNA polymerase (RdRp) protein sequences.[5] To date, 9 GI capsid genotypes and 19 GII capsid genotypes have been recognized in human; in addition, at least 14 GI polymerase (GI.P) genotypes and 27 GII.P genotypes have been described.[4] Since the mid-1990s, GII genotype 4 (GII.4) noroviruses have caused the majority of outbreaks and sporadic gastroenteritis worldwide.[6,7] Since 2002, new GII.4 variants have emerged every 2–3 years and replaced previous predominant GII.4 strains, resulting in epidemics and sometimes global pandemics of acute gastroenteritis.[7–9] GII.4 norovirus epochal evolution is driven by antigenic drift and recombination.[10] However, in the winter of 2014/2015, a novel GII.17 norovirus strain initially emerged in China and afterwards was found to circulated in other countries across 4 continents[11]; in late 2016, a new recombinant GII P16-GII.2 strain emerging in China replaced GII.17 as the predominant strain responsible for the majority of norovirus-associated outbreaks, and also was prevalent in Germany during the 2016–2017 season.[12–14]

Previous studies suggest certain epidemic GII.4 strains could be found in sporadic samples in both of these settings many years before becoming globally predominant and the majority of non-GII.4 strains causing the outbreak activity were inter-genotype recombinant viruses.[15] Thus, routine surveillance of sporadic norovirus infection is also important for understanding and predicting the emergence of epidemic strain and for selecting the potential vaccine strains. Based on the impact public health of norovirus and the current progress in norovirus vaccine development, it is necessary to continuously monitor the norovirus activity and circulating genotypes, especially in children who are more susceptible to norovirus and play an important role in driving transmission in the community.[16,17]

OBJECTIVE

The aim of this study was to update the recent molecular and clinical epidemiology of noroviruses and track the potential emergence of new strains and recombinants in pediatric diarrheal outpatients in Shanghai during 2014–2018.

STUDY DESIGN

Study Setting and Sample Collection

This surveillance study was conducted during 2014–2018 at the outpatient setting of Children's Hospital of Fudan University, the largest tertiary teaching pediatric hospital in Shanghai. Diarrhea

is defined as ≥3 abnormally loose or watery stools in previous 24 hours.[18] Watery diarrhea refers to diarrhea involves the passage of frequent loose or watery stools without visible blood.[19] The diarrheal outpatients who had onset of diarrhea symptoms 7 days before hospital visit and resided in Shanghai were enrolled twice a week. Stool samples were collected from the first 5–10 diarrheal cases whose fresh stool amount was adequate for stool microscope examination and virus detection. The stool samples were kept in a sterile container and frozen at −20℃ before RNA extraction.

This study was reviewed and approved by the Ethics Committee of Fudan Children's Hospital.

Identification of GI and GII Norovirus

Norovirus GI and GII genogroups were identified using one-step real-time reverse transcription-polymerase chain reaction (RT-PCR). In brief, 150 µl supernatant was taken from a 20% stool suspension for RNA extraction using Trizol (Invitrogen). Modified primer and probe sets based on sequences previously described were used.[20] 5′ flaps (AATAAATCATAA) were added to primers Cog 1F/Cog 1R and Cog 2F/Cog 2R to improve the fluorescent signal.[21] Probes Ring 1a/Ring 1b and Ring 2 were labeled with FAM and HEX at 5′ extremities, respectively. SuperScript III Platinum One-Step Quantitative Kit (Invitrogen) was used throughout the study. The final reaction volume was 25 µl consisting of 5 µl RNA and 20 µl RT-PCR master mix which was dispensed into the 96-well reaction plate. The quantitative RT-PCR was performed on the Applied Biosystems 7500 real-time PCR system (Applied Biosystems, CA). Amplification conditions followed the manufacturer's instruction. A negative control containing DEPC water and 2 positive controls containing RNA of norovirus GI and GII were included in each PCR run. Samples were scored as positive if cycle threshold values were ≤40 and positive and negative controls showed expected values.

Nucleotide Sequencing, Genotype Identification and Phylogenetic Analysis of GII Norovirus

First-strand cDNA synthesis of norovirus was performed using random primers with SuperScript III RT (Invitrogen) according to the manufacturer's protocol. PCR was carried out with KOD FX (Toyobo, Japan) using the Bio-Rad PCR system (Bio-Rad, CA). Primer sets JV12/JV13 were used to amplify 327 bp fragment of GII polymerase region.[22] Modified primer sets G2FF/G2SKR as described previously were used to amplify 468 bp for GII capsid region.[23] Twenty nucleotides (FSP: CAGGCCACGTTTTGTCATGCG and RSP: TTCTTTGCGTTATGTCTCTG) were added at the 5′ extremities of forward and reverse primers, respectively, to make the PCR products be used for direct sequencing.[21] The PCR cycling profile consisted of 95℃ for 2 minutes, 40 cycles of 98℃ for 10 seconds, 48℃ for 30 seconds, and 68℃ for 30 seconds, and then elongation for 7 minutes at 68℃. PCR products were purified from 1.5% agarose gels with a DNA extraction kit and amplicons were sequenced in both direction using FSP/RSP and JV12/JV13 on an ABI 3730 DNA Analyzer (Applied Biosystems).

Capsid genotypes were classified based on the 282 bp nucleotide sequences of the capsid N/S domain, consistent with the classification scheme of Kageyama et al[23]. Polymerase genotypes were classified based on the 327 bp nucleotide sequences of polymerase region. Genotypes of norovirus strains were determined by nucleotide sequence analysis using the online Norovirus Genotyping Tool Version 1.0 available at www.rivm.nl/mpf/norovirus/typingtool and confirmed using maximum likelihood method based on the Kimura 2-parameter model as implemented in Molecular Evolutionary Genetics Analysis software version 6.0.[24] Robustness of the trees was assessed using bootstrap analysis of 1000 replicates. The sequences of 45 local representative strains generated were submitted to GenBank.

Accession numbers for polymerase gene sequences are MK612114-MK612124, MK612132-MK612136, MK612399-MK612407, MK616564-MK616581, MK612760 and MK612110. The accessions numbers are MK603021-MK603026, MK603030-MK603052, MK593612 to MK593625, MK612111 and MK612113 for capsid gene sequences.

RESULTS

Prevalence and Seasonality of Noroviruses

Of the 3422 children with diarrhea, 510 (14.9%) were positive for noroviruses with 13 (2.5%) strains being GI genogroup and 497 (97.5%) strains being GII genogroup. The annual prevalence of norovirus-associated diarrhea was 12.6% (92/729) in 2014, 17.3% (137/790) in 2015, 17.0% (103/605) in 2016,15.5% (98/633) in 2017 and 12.0% (80/664) in 2018. Noroviruses were detected throughout a year and the increased activity of norovirus-associated diarrhea usually occurred in the autumn and winter seasons. However, the peak season and the duration of peak activity varied by year, as shown in Figure 1.

Demographics and Clinical Futures of Noroviruses-associated Diarrhea

In 3422 diarrheal outpatients between 1 month and 192 months (median age: 14.5 months) of age, 2121 (62.0%) were male and 1301 (38.0%) were female. In 510 norovirus-positive diarrheal outpatients between 1 month and 132 months of age (median age: 13.2 months), 344 (67.5%) were male and 166 (32.5%) were female; 131 (25.7%) had fever (defined as an elevation in core body temperature above normal values ≥37.9°C[25]); 260 (51.0%) had vomiting, and 158 (31.0%) had watery diarrhea.

The age-specific frequency of noroviruses in diarrheal outpatients for age group <6 months, 6–11 months, 1, 2, 3, 4 and ≥5 years old were 4.2% (21/505), 15.0% (160/1067), 19.9% (217/1093), 14.9% (40/268), 15.3% (25/163), 13.3% (11/83) and 14.8% (36/243), respectively. The prevalence of noroviruses was significantly lower in diarrheal children less than 6 months than in other age groups ($P < 0.05$).

The Distribution of Genotypes by Year

As shown in Table (Supplemental Digital Content 1, http://links.lww.com/INF/D611), 5 distinct capsid genotypes were identified for 348 norovirus GII strains successfully amplified, including

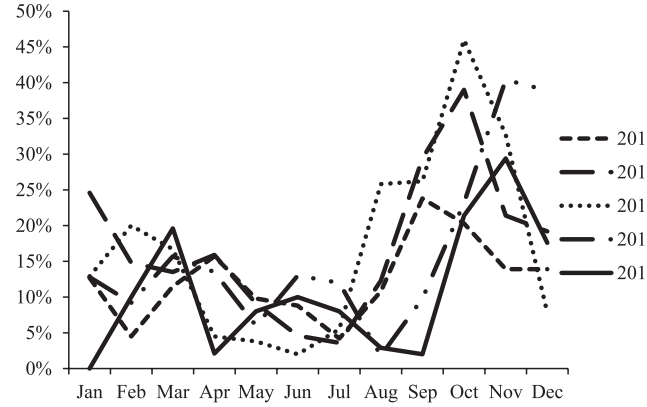

FIGURE 1. Seasonality of sporadic norovirus-associated diarrhea in Shanghainese children during 2014–2018.

250 (71.8%) GII.4- Sydney/2012, 48 (13.8%) GII.3, 27 (7.8%) GII.17, 21 (6.0%) GII.2, 1 (0.3%) GII.6 and 1 (0.3%) GII.8. GII.4-Sydney/2012 strains were predominantly prevalent in each year. Seven polymerase genotypes were identified for 335 norovirus GII strains successfully amplified, including 258 (77.0%) GII.Pe, 37 (11.0%) GII.P12, 30 (9.0%) GII.P17, 7 (2.1%) GII.P16, 1 (0.3%) GII.P8, 1 (0.3%) GII.P7 and 1 (0.3%) GII.P2. GII.P17 genotype was detected in consecutive years and more frequently prevalent in 2014 and 2015. GII.16 was detected since 2016 and more frequently prevalent in 2017.

Eleven dual polymerase and capsid genotype combinations were identified for 298 norovirus GII strains, including 221 (74.2%) GII.Pe/GII.4-Sydney/2012, 5 (1.7%) GII.Pe/GII.2, 3 (1.0%) GII.Pe/GII.3, 35 (11.7%) GII.P12/GII.3, 1 (0.3%) GII.P12/GII.4-Sydney/2012, 23 (7.7%) GII.P17/GII.17, 2 (0.7%) GII.P17/GII.4-Sydney/2012, 1 (0.3%) GII.P17/GII.3, 5 (1.7%) GII.P16/GII.2, 1 (0.3%) GII.P8/GII.8 and 1 (0.3%) GII.P7/GII.6. Three capsid genotypes were found to be associated with ≥1 polymerase genotype, including GII.4-Sydney/2012, GII.3 and GII.2.

The distribution of norovirus GII capsid and polymerase genotypes recombinant patterns by month in each year is shown in Figure 2. GII.Pe/GII.4-Sydney/2012, GII.P12/GII.3 and GII.P17/GII.17 strains were detected each year with GII.Pe/GII.4 being predominantly prevalent; the novel GII.P17/GII.17 variants were detected since September 2014 and detected year-round in 2015, then were detected occasionally in some months since 2016; the novel GII.P16/GII.2 recombinant variants emerged in December 2016 and were detected occasionally in some months in 2017 and 2018. Two recombinant GII.P17/GII.4-Sydney strain was detected in April 2014 and October 2017, and 1 recombinant GII.P17/GII.3 strains were detected in March 2016.

The peak seasonality for sporadic norovirus-associated diarrhea was primarily parallel with the highest prevalence of GII.4-Sydney/2012 genotypes.

Phylogenetic Analysis of Norovirus GII Genotypes

Forty-five Nov GII capsid/polymerase genotype recombinants were selected to construct phylogenetic trees and compared with the reference strains available in the GenBank databases (Figure, Supplemental Digital Content 2, http://links.lww.com/INF/D612). The GII.P17/GII.17 strains circulating in Shanghainese children were clustered together with GII.17 Japan strain (Hu/GII/JP/2015/GII.P17_GII.17/Kawasaki308; GenBank accession no. LC037415), GII.17 Guangdong strain (Hu/15F98/ZQ/GD/CHN/2015; GenBank accession no.KP718704.1), GII.17 Hong Kong strain (GII/Hu/HKG/2014/GII.17/CUHK-NS-463; GenBank accession no.KP998539) and Shanghai strain (152642/2015/Shanghai; GenBank accession no.KP864102), which shared the high similarity of the nucleotide sequences with identities of 98.9%–100%. The GII.P16/GII.2 circulating in Shanghainese children were clustered together with Taiwan GII.P16-GII.2 strain (GenBank accession no. KY457721), Guangdong GII.2 strain (accession no. KY485126), Germany GII.P16-GII.2 strain (GenBank accession no. KY357459) and the Hong Kong GII.2 strain sampled in 2016 (GenBank accession no. KY421044), which had 97.8%–99.0% identity in the nucleotide sequences. Phylogenetic analysis based on the partial capsid genes revealed that the GII.4 strains detected in 2014–2018 belonged to GII.4-Sydney 2012 genotype, sharing the highest identity with reference strain Australia NSW0514 (accession no. JX459908), Brazil VIR656F (accession no. MG023215), and Australia NSW028D (accession no. KT239579).

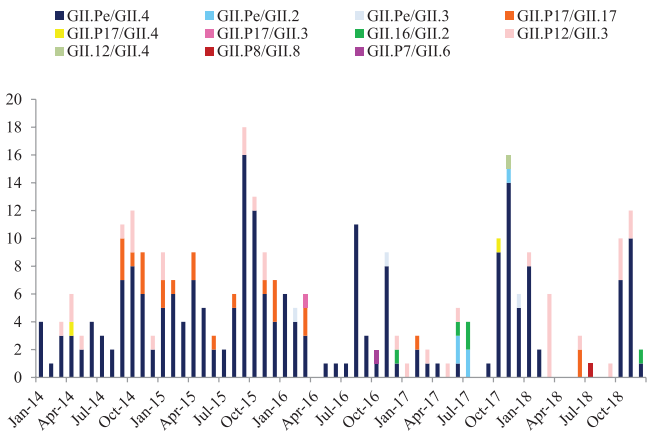

FIGURE 2. Number of norovirus GII polymerase/capsid genotypes with dual-typing results available by month from January 2014 to December 2018.

DISCUSSION

This study presents the latest epidemiologic pictures of norovirus infection in sporadic diarrhea in Shanghainese children. The average annual prevalence of norovirus-associated diarrheal was 14.9% during 2014–2018. Multiple norovirus capsid and polymerase genotypes co-circulated in Shanghai with predominance of GII.4-Sydney/2012 capsid genotype and GII.Pe polymerase genotype. To date, GII.4-Sydney 2012 has remained persistent in Shanghai without the novel GII.4 variant being found. The novel GII.P17/GII.17 strain emerging in the autumn of 2014 and the novel GII.P16/GII.2 recombinant strain emerging in the winter of 2016 were also detected in sporadic diarrheal children, which were reported to be associated with the increasing outbreaks of norovirus gastroenteritis in China and Japan during the winter of 2014/2015 and the winter of 2016/2017.[11–13,26–30]

This consecutive 5-year surveillance study shows that an annual prevalence of norovirus-associated diarrhea ranged from 12.0% to 17.3%. Norovirus has replaced rotavirus as the leading cause of pediatric gastroenteritis requiring medical attention in countries with a successful implementation of rotavirus vaccination, such as the United Sates, Finland and Nicaragua.[31–33] With the introduction of pentavalent rotavirus vaccine in China in 2018, norovirus will be expected to be the most common cause of diarrhea in young children in the future. Currently, candidate norovirus vaccines are in development for the need of public health and reducing the global disease burden.[34] Community-based studies revealed young children experience the highest overall incidence of norovirus diarrheal disease and also play an important role in driving transmission of norovirus in the community.[17,35,36] Accordingly, vaccinating young children would likely be most efficient for directly preventing disease burden, and be beneficial for indirectly reducing transmission. Additionally, we observed that the prevalence of norovirus-associated diarrhea significantly increased since 6 months of age. This result is helpful to schedule the time of norovirus vaccination for children. The high activities of sporadic norovirus diarrhea usually occurred in the autumn and winter seasons in Shanghai. The peak season of sporadic norovirus diarrhea usually appeared in autumn from September to November, which is earlier than in the northern China and other countries, where norovirus epidemics usually peak in the winter seasons.[7,37,38]

The novel GII.P17/GII.17 strain since September 2014 and the novel GII.P16/GII.2 recombinant strain since December 2016 emerged and circulated in sporadic diarrhea in Shanghainese children. However, GII.4 Sydney 2012 capsid genotype and GII.Pe polymerase genotype remained the most prevalent genotypes in Shanghainese children despite emergence of new non-GII.4 variants in

pediatric population. Nevertheless, the novel GII.P17/GII.17 and GII.P16/GII.2 strains replaced GII.4 strain as the most common genotypes causing acute diarrhea in adult patients.[26,39] We postulate that norovirus infection in adults is usually foodborne.[40] Both the polymerase-based tree and capsid-based phylogenetic trees showed that GII.P17/GII.17 strains circulating in Shanghainese children clustered closest to the first GII.17 Kawasaki isolate deposited in Genbank and formed a separate cluster from the GII.17 strains identified in 1978-2009. Lindesmith et al reported emergence and persistence of novel GII.17 strains in human populations correlate with change in blockade antibody epitopes.[41] The novel GII.P16/GII.2 recombinant variant was detected in Shanghainese children in December 2016. Both the polymerase-based tree and capsid-based phylogenetic trees showed that GII.P16/GII.2 strains circulating in Shanghainese children are closely related to the Germany GII.P16-GII.2 strains, a 2016 Japan GII.P16-GII.4 recombinant strain, and a Hong Kong GII.2 strain also sampled in 2016.

Emergence of GII.17 strains and GII.P16/GII.2 recombinant strains and 7-year persistence of GII.4-Sydney/2012 in Shanghainese children emphasizes the need for enhanced surveillance of norovirus to predict the potential changing trend of novel variants. Continuous monitoring of the predominant genotypes of norovirus in the population is also critical for designing the vaccine antigen formulations targeting the prevalent genotypes. Thus far, several norovirus vaccine candidates are in development and in early clinical trials.[34] An effective norovirus vaccine may require multiple constituent antigens as well as periodic reformulation to adapt to changes in molecular epidemiology. Circulating noroviruses are antigenically diverse and continually evolving, which challenges the development of an effective norovirus vaccine.

The findings of this study add the most recent epidemiologic data of norovirus from Chinese children, which will be useful to present a public health case in China and to guide interventions and norovirus vaccine development. It is necessary to monitor the prevalence and variation of norovirus strains for the development of prevention and control strategies.

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Current Abstracts

Edited by Robert J. Leggiadro, MD

The Epidemiology of Herpes Zoster in the United States During the Era of Varicella and Herpes Zoster Vaccines

Changing Patterns Among Children

Harpaz R and Leung JW. *Clin Infect Dis*. 2019; 69:345–347.

Varicella zoster virus (VZV) infection is a prerequisite for herpes zoster (HZ); a primary infection leads to varicella and, as it resolves, VZV establishes a latent infection in the dorsal root ganglia, from where it may reactivate to cause HZ years to decades later. The live-attenuated VZV contained in the varicella vaccine can also cause HZ; the risks appear to be less than those for wild-type VZV, but they remain incompletely defined.

While the epidemiology of HZ in adults has been changing for unrecognized reasons (Harpaz and Leung. Clin Infect Dis 2019; 69: 341–344), the varicella vaccination program may have had distinct, complex influences on the epidemiology of HZ among children. The results of an analysis of HZ trends in children from the years 1998 to 2016 are reported.

Analysis is based on IBM MarketScan Research Databases for the years 1998 to 2016. These databases include data from public and private employers, health insurance plans and Medicare for individuals residing in all states. Incident HZ cases were defined using outpatient and emergency department claims with ICD-9/10 diagnostic codes for HZ in the primary or secondary diagnostic position. For a given person, only the first HZ episode during the study period was included. HZ trends were analyzed using data on children <18 years of age; a secondary analysis of age-specific HZ rates included persons <35 years of age.

There were a total of 13,084,793 children <18 years of age enrolled between the 1998 and 2016 study interval; 35,405 incident HZ cases were coded in the primary diagnostic position. HZ incidence changed in each age stratum, showing increases followed by decreases in a step-like manner. The peak incidence occurred in progressively earlier calendar years among progressively younger strata, with all occurring among cohorts born approximately between 1990 and 1996; the incidences declined from peak values by about 70%–80% in the different age cohorts, with absolute values as low as 0.22 per 1000 among children in each of the 2 youngest age strata. For each age stratum, differences in HZ incidences by sex appeared to sharply converge as the incidences declined. The HZ incidences increased by age during childhood and early adulthood in individuals born prior to 1996, but age-specific HZ incidences flattened out for later birth cohorts.

Comment: Prior to the licensure of the varicella vaccine in the United States in 1996, almost the entire population experienced infection with the VZV, causing varicella. As the varicella vaccination program was introduced and was maturing (a second dose was recommended in 2006), HZ incidences have declined dramatically among children since 1998, according to this report. These results suggest that, over decades, this declining HZ incidence will extend to progressively older adults, ultimately extending to the entire population.