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Norovirus outbreaks in Beijing, China, from 2014 to 2017

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SUMMARY

Objectives: Noroviruses are a leading cause of acute gastroenteritis (AGE) outbreaks worldwide. This study examined the epidemiology and genetic characteristics of norovirus outbreaks in Beijing, China.
Methods: Epidemiological data and fecal specimens were collected through the AGE outbreak surveillance system in Beijing. GI and GII genogroup noroviruses were detected and genotyped. The data were analyzed using descriptive statistics.
Results: Between September 2014 and August 2017, 762 AGE outbreaks were reported in Beijing, of which 661 (86.7%) were laboratory-confirmed as norovirus. Most norovirus outbreaks were reported during the spring (66.9%, 442/661), occurred in kindergartens and elementary schools (92.3%, 610/661), and were caused by GII genogroup noroviruses (95.6%; 632/661). The genotypes of the norovirus strains were determined in 468 outbreaks, and GII.P16-GII.2 and GII.P17-GII.17 strains were the most commonly identified. GII.P17-GII.17 and GII.P16-GII.2 strains predominated in 2014–2015 and 2016–2017 outbreaks, respectively. GII.P16-GII.2 noroviruses were responsible for a steep increase in AGE outbreaks in Beijing: 549 norovirus outbreaks were reported from 2016 to 2017, 9.2 times the number that occurred during the previous year.
Conclusions: Norovirus causes a large disease burden in Beijing, and the prevalence of non-GII.4 noroviruses presents a new challenge for the development of vaccines.

Introduction

Noroviruses are a leading cause of sporadic cases and outbreaks of acute gastroenteritis (AGE) worldwide.[1] Norovirus outbreak surveillance mechanisms are already established in developed countries, where about 50% of all outbreaks of AGE occur.[2] However, in developing countries including China, most studies have focused on sporadic AGE, and the epidemiological features of norovirus outbreaks remain unclear.

Norovirus AGE generally has an incubation period of 24–48 h and is characterized by acute onset of nausea, vomiting, abdominal cramps and diarrhea. This disease is usually self-limiting and symptoms typically resolve with in 2–3 days[3,4]. Norovirus can be transmitted via the fecal-oral cycle through the ingestion of contaminated food or water or through direct contact with contaminated environmental surfaces or infected persons. Exposure to noroviruses in aerosolized vomitus has also been linked with infection[5]. In developed countries, the majority of norovirus outbreaks occur in healthcare facilities such as long-term care facilities (LTCFs) and hospitals[6].

Noroviruses are single-stranded positive-sense RNA viruses, with genomes approximately 7.3–7.5 kb in length. The norovirus genome is organized into three open reading frames (ORFs): ORF1 encodes a large polyprotein, which is cleaved into at least six mature nonstructural proteins including RNA-dependent RNA polymerase (RdRp). ORF2 and ORF3 encode the major (VP1) and minor (VP2) capsid proteins, respectively.[7] Noroviruses are classified into seven genogroups (I-VII), which are subdivided into 39 genotypes on the basis of their complete VP1 amino acid sequences[8]. Genogroup II genotype 4 (GII.4) strains have been responsible for the majority of outbreaks and sporadic cases of AGE since the mid-1990s. New GII.4 variants emerge every 2–3 years, causing global AGE epidemics[8]. However, during the winter of 2014–2015, a novel GII.17 norovirus replaced the Sydney 2012 GII.4 virus as the predominant strain in East Asia[9].

Mutation and recombination are the major forces driving norovirus evolution, and the most commonly identified hotspot for genome recombination lies at the ORF1-ORF2 junction region[10].

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Between 2004 and 2012, approximately 70% of reported recombinant noroviruses identified in children with sporadic AGE worldwide were GII.Pb-GII.3, GII.P12-GII.3 and GII.P4-GII.3, and GII.P12-GII.3 was the predominant recombinant strain in Asian countries including Japan, China and Korea[11].

Since 2011, a hospital-based surveillance network for sporadic AGE caused by norovirus and rotavirus has been established in all 16 districts of Beijing, China. To enhance AGE surveillance, the Beijing Center for Disease Control and Prevention (CDC) and its district partners developed an AGE outbreak surveillance network in April 2014. In this study, we describe the epidemiological and genetic features of norovirus outbreaks in Beijing from September 2014 to August 2017.

Methods

AGE outbreak surveillance

Cases of AGE were defined as patients with diarrhea (three or more loose stools within a 24 h period) and/or vomiting (one or more episodes). An outbreak was defined as the occurrence of three or more epidemiologically-linked cases of AGE within a period of 3 days. Most AGE outbreaks were reported to the community health centers by local schools, kindergartens and other institutions, and the community health centers reported them to district-level CDCs. A few outbreaks were reported to district-level CDCs by public complaints and hospital clinics. District-level CDCs finished the preliminary investigation within 24 h of receipt of the report, and fecal specimens were collected from cases within 48 h of initiation of the investigation. During the investigations, norovirus and other enteric pathogens were detected. A norovirus outbreak was confirmed if more than two AGE cases tested positive for norovirus by real-time reverse transcription polymerase chain reaction (RT-PCR).

The transmission modes of norovirus outbreak include foodborne, water-borne and person-to-person. Norovirus is highly contagious and person-to-person transmission occurs in most outbreaks. In this study, the transmission mode was referred to the main transmission mode in one outbreak, and the subsequent person-to-person transmission was not included. The general mode of transmission was identified using the descriptive epidemiological study, Analytical epidemiological studies, including cohort and case-controls, were performed to identify specific exposures.

Norovirus-positive specimens were sent to the Beijing CDC and 2–5 specimens per outbreak were selected for sequencing. In this study, a norovirus outbreak surveillance year was defined as starting on 1 September and ending on 31 August of the following year.

Viral RNA extraction and norovirus detection

Viral RNA was extracted from 140 µL of a 10% (w/v) fecal suspension in phosphate-buffered saline using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. RNA was stored at −20°C until further use.

The presence of GI and GII genogroup noroviruses was detected using a SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen, Carlsbad, CA) and previously-described primers and probes.[12]

RT-PCR

The QIAGEN One-Step RT-PCR Kit (QIAGEN, Hilden, Germany) was used to amplify norovirus genes in a 50-µL reaction volume. RNase Inhibitor (Promega, Madison, WI, USA) was added at a final concentration of 5–10 units/reaction. Between September 2014 and August 2016, RT-PCRs for genotyping were performed as described previously[9]. Between September 2016 and August 2017, a novel RT-PCR assay targeting regions B and C of the norovirus genome was performed with primers MON432/G1SKR for GI viruses and primers MON431/G2SKR for GII viruses, yielding 579-bp and 570-bp PCR products[13–15]. RT-PCR reactions were incubated at: (i) 50 °C for 30 min, (ii) 95 °C for 15 min, (iii) 40 cycles of 95 °C for 60 s, 54 °C for 60 s and 72 °C for 60 s, and (iv) 72 °C for 10 min. PCR products were analyzed using a QIAxcel Advanced Instrument with a QIAxcel DNA Screening Kit (QIAGEN, Hilden, Germany).

DNA sequencing and phylogenetic analysis

PCR products were purified and sequenced directly on an ABI 3730xl DNA Analyzer using a BigDye Terminator v3.1 Cycle Sequencing Kit (ABI, Austin, TX, USA). All sequences were prepared and aligned using BioEdit (version 7.0.9.0) and Clustal W. Genotypes were determined by phylogenetic analysis or/and the Norovirus Typing Tool (available at http://www.rivm.nl/mpf/norovirus/typingtool). A phylogenetic tree was constructed using the maximum likelihood method implemented in MEGA software (version 6.06) with 1000 bootstrap replicates. The sequences of the noroviruses identified in this study were deposited in GenBank (accession nos. KY635865–KY635875 and MF372824–MF372831).

Data analyses

Epidemiological, clinical, and genotyping data for all AGE outbreaks were collected and imported into WPS Spreadsheets 2016 (Kingsoft Inc., Beijing, CHN) for basic data manipulation and graphing. Statistical analyses were performed using SPSS v19.0 software (SPSS Inc., Chicago, IL, USA). The chi-square test was used to compare the differences in epidemiological characteristics between norovirus outbreaks caused by different genogroups or genotypes. The Cochran-Armitage trend test was used to analyze correlations between symptoms and patient age. The Wilcoxon signed rank test was used to analyze differences in the ages of patients infected by different norovirus genotypes. Statistical significance was assumed for $p < 0.05$.

Ethics statement

This study was approved by the Ethics Committee of the Beijing CDC.

Results

Epidemiological characteristics of norovirus outbreaks in Beijing, China, from 2014 to 2017

Between September 2014 and August 2017, 762 AGE outbreaks were reported in Beijing, of which 661 (86.7%) were laboratory-confirmed as norovirus. The median number of individuals affected per outbreak was 11 (range: 3–172) and the median duration of outbreak was 3 days (range: 1–20 days). Attack rates were calculated for 656 norovirus outbreaks and ranged from 0.9% to 80.8% with a median of 24.8%. In total, 52 norovirus outbreaks were reported from 2014 to 2015, 60 norovirus outbreaks were reported in 2015 to 2016, and 549 norovirus outbreaks were reported from 2016 to 2017; the lattermost figure represented 9.2 times the number of outbreaks in the previous year. In 2014–2015, the majority of norovirus outbreaks (55.8%, 29/52) occurred in October, January and March. By contrast, 66.7% (40/60) of the 2015–2016 outbreaks and 86.7% (476/549) of the 2016–2017 outbreaks occurred between March and June (Fig. 1). Norovirus outbreaks were reported in all 16 districts of Beijing, with 408 (61.7%) occurring in urban areas (Chaoyang, Haidian, Fengtai, Docheng, Shijingshan and Xicheng), 217 (32.8%) occurring in suburbs (Tongzhou, Daxing, Changping,

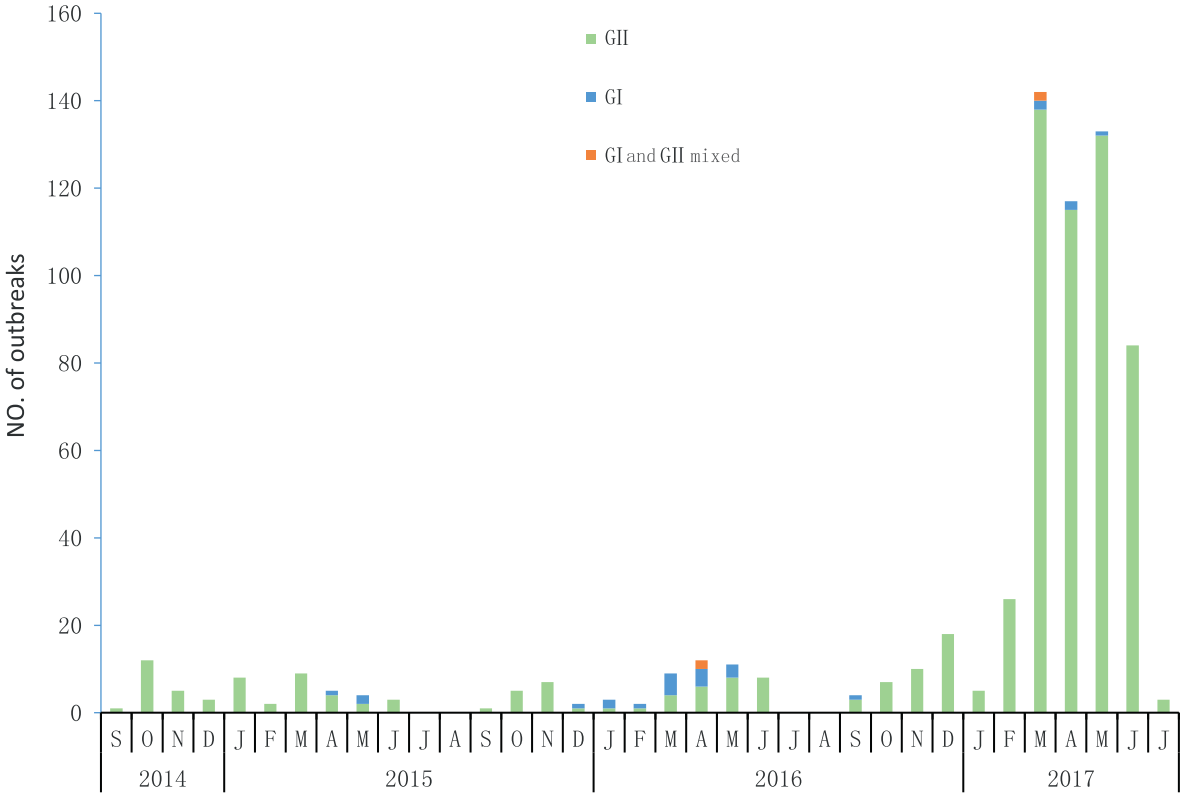

Fig. 1. Monthly reported norovirus outbreaks in Beijing, China, between September 2014 and August 2017.

Table 1
Clinical features of patients involved in norovirus outbreaks.

Age (years)	Fever	Vomiting	Diarrhea
1–6	33.49% (1406/4198)	99.02% (4157/4198)	5.69% (239/4198)
7–17	25.01% (1283/5129)	93.76% (4809/5129)	17.06% (875/5129)
≥18	23.54% (125/531)	73.63% (391/531)	58.38% (310/531)
χ^2	88.232	659.534	1117.944
p value	<0.001	<0.001	<0.001

Shunyi, Fangshan and Mentougou), and 36 (5.4%) occurring in outer suburbs (Miyun, Huairou, Pinggu, Yanqing). Most norovirus outbreaks occurred in kindergartens (343, 51.9%) and elementary schools (267), although outbreaks also occurred in middle schools (31), colleges (7), nine-year schools (4), families (2), government agencies (2), a company (1), a factory (1) a hospital (1), a touring party (1) and a building site (1). The transmission modes were determined for 600 outbreaks. The most common transmission mode was person-to-person (589/600, 98.2%) followed by food-borne transmission (11/600, 1.8%).

Clinical features of patients involved in norovirus outbreaks

A total of 9858 individuals were affected by reported norovirus outbreaks with a median age of 7 years (range: 1.5–84 years). The gender ratio (male/female) was 1.2. All cases were mild and there were no hospitalizations or deaths, although 45.3% (4470/9858) of patients had seen their doctor. All cases reported symptoms, with the most common symptoms being vomiting (94.9%, 9357/9858), fever (28.5%, 2814/9858) and diarrhea (14.4%, 1424/9858). The frequency of vomiting and fever symptoms decreased with advancing age ($p < 0.001$, Cochran-Armitage trend test), while the frequency of diarrhea increased with advancing age ($p < 0.001$, Cochran-Armitage trend test) (Table 1). Most cases (85.6%, 8434/9858) reported vomiting without diarrhea, while 5.1% (501/9858) reported diarrhea without vomiting, 60.9% (5999/9858) reported vomiting only and 4.4% (435/9858) reported diarrhea only.

Norovirus genotypes

Of the 661 norovirus outbreaks, 25 (3.8%) were caused by GI genogroup noroviruses, 632 (95.6%) were caused by GII genogroup noroviruses, and 4 (0.6%) were caused by a combination of GI and GII genogroup noroviruses. Norovirus genomes were sequenced in 468 outbreaks and GII.P16-GII.2 was the most frequently detected genotype (70.1%, 328/468). Other genotypes that caused 1% or more outbreaks included GII.P17-GII.17 (8.8%, 41/468), GII.P12-GII.3 (5.1%, 24/468), GII.P7-GII.6 (3.4%, 16/468), GII.P2-GII.2 (2.6%, 12/468), GI.Pb-GI.6 (2.6%, 12/468), GII.Pe-GII.4 Sydney 2012 (2.1%, 10/468) and mixed outbreaks involving more than one genotype (1.9%, 9/468) (Table 2). GII.P17-GII.17 genotypes predominated in outbreaks during 2014–2015. No predominant strain was responsible for outbreaks during 2015–2016, although GII.P12-GII.3 (27.1%, 13/48), GII.P7-GII.6 (18.8%, 9/48) and GI.Pb-GI.6 (18.8%, 9/48) were the major causative genotypes. GII.P16-GII.2 genotype predominated in 2016–2017. The GII.P16-GII.2 strain emerged in Beijing in October 2016 and had been the predominant strain between February and July 2017 (Fig. 2).

Molecular epidemiology of norovirus outbreaks

The median numbers of individuals involved in GII and GI genogroup outbreaks were 10 (range: 3–172) and 15 (range: 6–43), respectively. GII genogroup outbreaks dominated in all reported settings and were most common in kindergartens, while GI outbreaks were most common in primary schools. The median (IQR) age of GII cases ($n = 9382$, median 7 years, interquartile range 5–9 years) was younger than that of GI cases ($n = 421$, median 8

Table 2
Number and percentage of outbreaks by genotype and by year.

Genotype	No. of outbreaks (%)			Total (%)
	2014–2015	2015–2016	2016–2017	
GI.P2-GI.2	0 (0)	1 (2.1)	1 (0.3)	2 (0.4)
GI.P3-GI.3	0 (0)	2 (4.2)	0 (0)	2 (0.4)
GI.P6-GI.6	0 (0)	1 (2.1)	0 (0)	1 (0.2)
GI.Pb-GI.4	0 (0)	1 (2.1)	0 (0)	1 (0.2)
GI.Pb-GI.6	3 (7.7)	9 (18.8)	0 (0)	12 (2.6)
GI.Pd-GI.3a	0 (0)	0 (0)	1 (0.3)	1 (0.2)
GII.P2-GII.2	2 (5.1)	4 (8.3)	6 (1.6)	12 (2.6)
GII.P7-GII.6	6 (15.4)	9 (18.8)	1 (0.3)	16 (3.4)
GII.P7-GII.7	2 (5.1)	0 (0)	1 (0.3)	3 (0.6)
GII.Pe-GII.4 Sydney 2012	0 (0)	3 (6.3)	7 (1.8)	10 (2.1)
GII.Pg-GII.1	0 (0)	1 (2.1)	1 (0.3)	2 (0.4)
GII.P12-GII.3	0 (0)	13 (27.1)	11 (2.9)	24(5.1)
GII.P15-GII.15	0 (0)	0 (0)	2 (0.5)	2 (0.4)
GII.P16-GII.2	0 (0)	0 (0)	328 (86.1)	328 (70.1)
GII.P16-GII.4 Sydney 2012	0 (0)	0 (0)	1 (0.3)	1 (0.2)
GII.P17-GII.17	26 (66.7)	4 (8.3)	11 (2.9)	41 (8.8)
GII.P20-GII.20	0 (0)	0 (0)	1 (0.3)	1 (0.2)
Mixed	0 (0)	0 (0)	9 (2.4)	9 (1.9)
Total	39 (100)	48 (100)	381 (100)	468 (100)

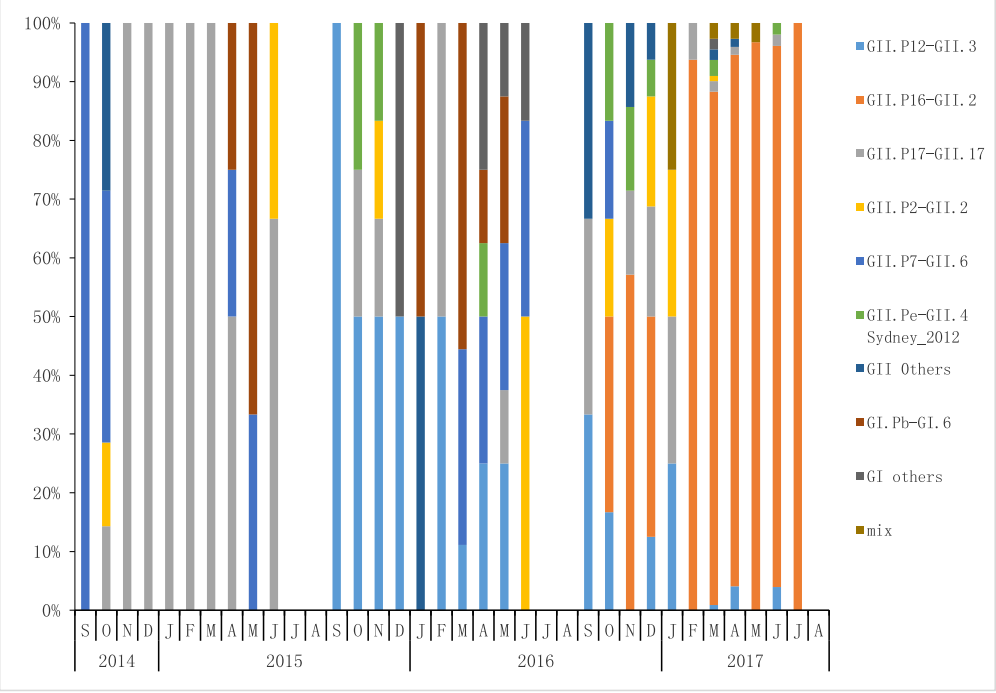

Fig. 2. Monthly genotype distribution of norovirus outbreaks in Beijing, China, between September 2014 and August 2017.

years, interquartile range 6–10 years) ($p = 0.029$, Wilcoxon signed rank test). GII cases also reported the higher frequencies of fever and lower frequencies of vomiting. There were no significant differences in gender of infected individuals, seasonality, regional distribution and transmission route between GII and GI outbreaks (Table 3).

The outbreaks caused by GII.P16-GII.2 and GII.P17-GII.17 strains (the main genotypes identified in this study) were compared (Table 4). These strains both affected a median of 12 patients per outbreak (range: 3–108 for GII. P16-GII.2 and 3–172 for GII.P17-GII.17). Most GII.P16-GII.2 outbreaks were reported in spring (76.8%), whereas GII.P17-GII.17 outbreaks occurred in both winter (36.6%) and spring (34.1%). GII.P16-GII.2 outbreaks mainly occurred in urban (54.9%) and suburban areas (40.2%), while GII.P17-GII.17 outbreaks were more concentrated in urban areas (75.6%). Though kindergartens were most common settings in both strains, the higher proportion of primary school outbreaks were caused by GII.P16-GII.2 strains than by GII.P17-GII.17 strains. Person to person was the main transmission mode for both genotypes, but food-borne transmission was more common in GII.P17-GII.17 outbreaks. The median age of GII.P16-GII.2 cases ($n = 5114$, median 7 years, interquartile range 5–9 years) was younger than that of GII.P17-GII.17 cases ($n = 921$, median 8 years, interquartile range 5–18 years) ($p < 0.0001$, Wilcoxon signed rank test). There was no significant difference in gender between the individuals infected by these two genotypes. GII.P16-GII.2 cases had higher frequencies of vomiting and the lower frequencies of diarrhea than GII.P17-GII.17 cases.

Phylogenetic analysis of GII.P16-GII.2 noroviruses

A phylogenetic tree was constructed based on partial RdRp and VP1 gene sequences (Fig. 3). GII.P16-GII.2 noroviruses formed three clusters: GII.P16-GII.2 2016–2017, GII.P16-GII.2 2010–2012, and GII.P16-GII.2 2008–2015 (Fig. 3). The GII.P16-GII.2 noroviruses

Table 3
Comparison of epidemiological characteristics between GI and GII genogroup norovirus outbreaks.

Groups	GI	GII	χ^2	*p* value
No. of outbreaks	25	632		
Median no. of patients per outbreak	15 (6–43)	10 (3–172)		
Season			5.821	0.121
Spring	20 (80%)	418 (66.1%)		
Summer	0 (0%)	98 (15.5%)		
Autumn	1 (4%)	51 (8.1%)		
Winter	4 (16%)	65 (10.3%)		
Region			0.443	0.801
Urban area	17 (68%)	389 (61.6%)		
Suburbs	7 (28%)	208 (32.9%)		
Outer suburbs	1 (4%)	35 (5.5%)		
Settings			8.518	0.036
Kindergarten	7 (28%)	335 (53%)		
Primary school	17 (68%)	249 (39.4%)		
Middle school	1(4%)	29 (4.6%)		
Others	0 (0%)	19 (3%)		
Transmition mode			0.673	0.714
Person to person	22 (88%)	564 (89.2%)		
Foodborne	(0%)	11 (1.7%)		
Unknown	3 (12%)	57 (9%)		
No. of patients involved	421	9382		
Symptoms of patients				
Vomiting	410 (97.4%)	8900 (94.9%)	5.377	0.02
Diarrhea	60 (14.3%)	1341 (14.3%)	0.001	0.981
Fever	72 (17.1%)	2722 (29%)	28.051	<0.001
Age of patients (years)			52.272	<0.001
2-6	109 (25.9%)	4080 (43.5%)		
7-17	288 (68.4%)	4804 (51.2%)		
⩾18	24 (5.7%)	498 (5.3%)		
Gender of patients (male)	215 (51.1%)	5057 (53.9%)	1.3	0.254

Table 4
Comparison of epidemiological characteristics between GII.P17-GII.17 and GII.P16-GII.2 genotype norovirus outbreaks.

Groups	GII.P16-GII.2	GII.P17-GII.17	χ^2	*P*-Value
No. of outbreaks	328	41		
Median no. of patients per outbreak	12 (3–108)	12 (3–172)		
Season			81.044	<0.001
Spring	252 (76.8%)	14 (34.1%)		
Summer	49 (14.9%)	3 (7.3%)		
Autumn	6 (1.8%)	9 (22%)		
Winter	21 (6.4%)	15 (36.6%)		
Region			6.401	0.041
Urban area	180 (54.9%)	31 (75.6%)		
Suburbs	132 (40.2%)	9 (22%)		
Outer suburbs	16 (4.9%)	1 (2.4%)		
Settings			33.316	<0.001
Kindergarten	171 (52.1%)	19 (46.3%)		
Primary school	138 (42.1%)	11 (26.8%)		
Middle school	13 (4%)	3 (7.3%)		
Others	6 (1.8%)	8 (19.5%)		
Transmition mode			67.79	<0.001
Person to person	313 (95.4%)	24 (58.5%)		
Foodborne	2 (0.6%)	6 (14.6%)		
Unknown	13 (4%)	11 (26.8%)		
No.of patients involved	5114	921		
Symptoms of patients				
Vomiting	4891 (95.6%)	834 (90.6%)	41.425	<0.001
Diarrhea	672 (13.1%)	224 (24.3%)	77.174	<0.001
Fever	1492 (29.2%)	263 (28.6%)	0.145	0.703
Age of patients (years)			932.025	<0.001
2–6	2090 (40.9%)	280 (30.4%)		
7–17	2918 (57.1%)	382 (41.5%)		
⩾18	106 (2.1%)	259 (28.1%)		
Gender of patients (male)	2759 (53.9%)	469 (50.9%)	2.874	0.09

identified in this study belonged to the GII.P16-GII.2 2016–2017 clade, which also included strains detected in Germany, Japan, France, Australia, the United States, Russia and other regions of China in 2016–2017. GII.P16-GII.2 2016–2017 strains had a closer genetic relationship with the GII.P16-GII.2 2010–2012 clade, sharing 96.3–98.6% sequence identity. GII.P16-GII.2 2010–2012 strains were detected in Japan, Singapore and the United States from 2010 to 2012. GII.P16-GII.2 2016–2017 strains shared 95.4–96.9% sequence identity with GII.P16-GII.2 2008–2015 strains, which were identified over a longer time range.

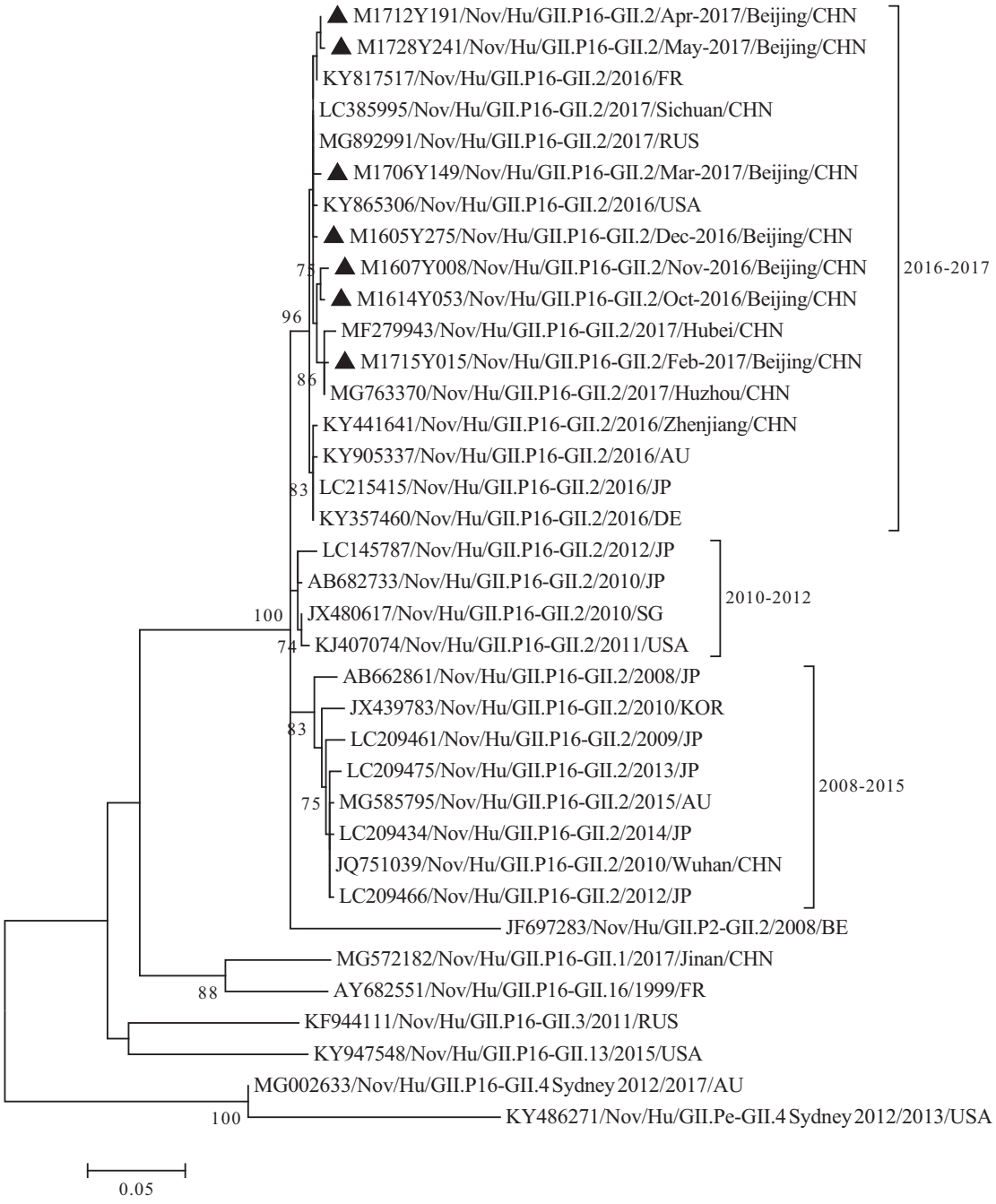

Fig. 3. Phylogenetic analysis based on partial RdRp and VP1 genes (527 bp) of GII.P16- GII.2 noroviruses. Norovirus strains detected in this study were marked with the following symbols: ▲. The reference sequences were retrieved from GenBank. The trees were generated using the maximum likelihood method with the nucleotide substitution model Kimura 2-parameter + Invariant sites. Bootstrap values, estimated from 1000 replicates, are indicated at each node. The scale bar indicates the number of nucleotide substitutions per site.

Discussion

A sensitive monitoring system for norovirus outbreaks has been established in Beijing, China since 2014. In Beijing, an AGE outbreak is defined as the occurrence of three or more epidemiologically-linked cases within a period of 3 days. By contrast, in most provinces of China, outbreaks are often defined as more than 20 cases per week in a community, and only AGE outbreaks meeting this definition are reported. In this study, 661 norovirus outbreaks were confirmed, and most involved fewer than 20 cases. Thus, it is possible that a large number of outbreaks were not reported in other provinces, and norovirus may cause a larger burden of disease in China than indicated by surveillance data.

In the temperate northern hemisphere, norovirus-associated AGE mainly occur during the cool months, with a peak in the winter[16]. During 2014–2015, the occurrence of norovirus outbreaks in Beijing was consistent with this general pattern. However, 66.7% of the 2015–2016 outbreaks and 86.7% of the 2016–2017 outbreaks occurred between March and June. Especially, a huge increase in norovirus outbreaks was observed in Beijing between February and June 2017, thus differing from the usual seasonality. According to meteorological standards, the first days of spring and summer in

Beijing in 2017 were identified as March 26th and May 7th, respectively, and the temperature had begun to rise after April 2017. A previous study in England and Wales highlighted that increased norovirus activity was associated with cold, dry temperature, low population immunity and the emergence of novel variants[17]. The temperature was not cold and dry in Beijing between April and July 2017; however, a new GII.P16-GII.2 variant predominated during this period. Since GII.2 viruses were rarely detected in Beijing prior to 2016[18,19,20], the population may lack protective immunity against this genotype. Thus, both low population immunity and the emergence of a new variant may have been responsible for this increase in outbreaks, and could have influenced the seasonality of norovirus activity.

Norovirus outbreaks mainly occurred in kindergartens and primary schools in Beijing, affecting children under 12 years of age; similar results were observed in other regions of China[21]. However, norovirus outbreaks mainly occurred in healthcare facilities such as LTCFs and hospitals in developed Western countries[6]. These inconsistent results have at least two explanations. First, different social cultures exist in these two regions: care of the elderly is given mainly by family members in China and few LTCFs are in use, whereas these are common in the United States. Second, major differences exist in the sensitivity and coverage of surveillance systems between regions. In the United States, outbreak reporting from institutions other than LTCFs was not legally mandated, and thus outbreaks in these settings were underreported[22]. In England, in addition to the national surveillance system for general outbreaks of infectious intestinal disease, a dedicated hospital norovirus outbreak reporting system was launched in 2009. More norovirus outbreaks were reported in the system's first full season than in the preceding 17 years (1884 vs 1817)[23]. In Beijing, reporting of infectious diseases is better performed in kindergartens and primary schools than other institutions; outbreaks of nosocomial infections are managed by other government departments and are usually not reported to the CDC.

In this study, the most common symptom reported by norovirus-infected individuals was vomiting (94.9%), and only 14.4% of individuals experienced diarrhea symptoms. Most cases (85.5%) reported vomiting without diarrhea. Most patients considered their symptoms to be mild and did not visit their doctor. Most studies define a case of norovirus infection based on the presence of diarrhea. Our findings suggested that a diarrhea-only case definition will result in a significant underestimate of the true prevalence of norovirus AGE. The average patient sheds 1.7×10^8 viral genomic copies/mL in emesis[24]. Unlike shedding through stool, vomiting is more likely to result in significant environmental contamination, leading to transmission through fomites and airborne droplets. Vomiting symptoms should be given more attention in future norovirus studies.

GII outbreaks were most common in kindergartens whereas GI outbreaks mainly occurred in primary schools. Thus, the individuals involved in GI outbreaks were older than those involved in GII outbreaks. GII outbreaks occurred during spring, autumn and winter, while GI outbreaks mainly occurred in the spring, which suggests that climate may impact norovirus genotype distribution.

Over the past two decades, norovirus GII.4 strains have been responsible for the majority of AGE outbreaks worldwide.[2] However, GII.P17-GII.17 strains predominated in China during the 2014–2015 season, ending the monopoly of GII.4 strains[25]. GII.P16-GII.2 strains caused a significant increase in the number of norovirus outbreaks in Beijing during the 2016–2017 season, far higher than the number of outbreaks caused by GII.P17-GII.17 strains during the 2014–2015 season. This suggests that GII.P16-GII.2 strains have superior epidemiological fitness in Beijing than GII.P17-GII.17 viruses. In the winter of 2016 and 2017, GII.P16-GII.2 viruses became the predominant genotype and caused the increased norovirus activity in other provinces of China, Germany, and France (where they caused 14.0% of norovirus outbreaks)[21,26–29]. By contrast, GII.P17-GII.17 strains only predominated in East Asia in 2014–2015[30]. These data suggest that new GII.P16-GII.2 strains have wider epidemic range than GII.P17-GII.17 strains.

Except for during autumn, GII.P16-GII.2 strains caused a higher number of outbreaks than GII.P17-GII.17 strains in this study. Most GII.P17-GII.17 outbreaks were reported in winter and spring, similar to GII.4 strains, but GII.P16-GII.2 outbreaks mainly occurred in the spring and summer. GII.P17-GII.17 outbreaks were concentrated in urban areas, while GII.P16-GII.2 outbreaks had a larger epidemic range, covering urban and suburban areas. Person to person transmission was more common in GII.P16-GII.2 outbreaks than in GII.P17-GII.17 outbreaks. These differences suggested that GII.P16-GII.2 strains have higher transmissibility and affected more susceptible individuals, and may be more tolerant to climate factors such as temperature and humidity. Compared with cases infected with GII.P17-GII.17 strains, cases infected with new GII.P16-GII.2 strains had a higher frequency of vomiting and a lower frequency of diarrhea. This may be associated with the age of infected individuals: the individuals involved in GII.P16-GII.2 outbreaks were younger than those involved in GII.P17-GII.17 outbreaks. The frequencies of vomiting gradually decreased with advancing age and the opposite trend was observed for diarrhea in this study. A previous study had also found that vomiting occurred more frequently in children and that diarrhea was more common in adults with norovirus infection.[5]

Phylogenetic analysis showed GII.P16-GII.2 noroviruses formed three clusters: GII.P16-GII.2 2016–2017, GII.P16-GII.2 2010–2012, and GII.P16-GII.2 2008–2015. The separate clusters suggested that GII.P16-GII.2 noroviruses were highly diverse and GII.P16-GII.2 2016–2017 strains were new variants. Such genetic diversity enabled these viruses to adapt to the environment and maintain the survival. GII.P16-GII.2 2016–2017 strains had a closer genetic relationship with GII.P16-GII.2 2010–2012 strains, suggesting that they diverged from a common ancestor. The VP1 amino acid sequences of new GII.P16-GII.2 strains were almost identical to those of previously detected GII.2 viruses, and their antigenicity and histo-blood group antigen binding profiles were also similar[31,32]. Compared with previous GII.P16 strains, five or four amino acid mutations were observed in the RdRp genes of new GII.P16-GII.2 strains[31,33], which may be associated with higher transmissibility of these GII.2 viruses, as observed for GII.P16-GII.4 Sydney 2012 viruses[34]. Global analyses found that GII.2 strains only accounted for 1.2% of all norovirus strains in children ($\leq$18 years of age) with sporadic AGE between 2004 and 2012[11]. They also only accounted for $<$1.2% of all norovirus sequences from China prior to 2016[35,36]. Therefore, most people may lack immune protection against GII.2 strains. These two factors may have contributed to the sharp increase in norovirus outbreaks in China in 2017.

Norovirus causes a large burden of disease in Beijing, especially when new variants appear, so ongoing surveillance and timely interventions are needed. GII.4 noroviruses have been responsible for the majority of outbreaks and sporadic cases of AGE worldwide, and therefore most norovirus studies have focused on this genotype. Since late 2014, the predominant norovirus genotype changed from GII.Pe-GII.4 Sydney 2012 to GII.P17-GII.17 and then to GII.P16-GII.2 in China, causing an increase in AGE outbreaks in the winters of 2014–2015 and 2016–2017. This highlights the complexity of norovirus gene variation and presents a new challenge for the development of norovirus vaccines.

Conflict of interest

The authors declare no conflicts of interest.

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