

## Preplanned Studies

## Asymptomatic Norovirus Infection Among Children in Kindergartens and Primary Schools — Beijing Municipality, China, 2021

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### Summary

#### What is already known about this topic?

Children in kindergartens and primary schools are the high-incidence groups of norovirus acute gastroenteritis. However, asymptomatic norovirus infection among them is seldom reported.

#### What is added by this report?

The norovirus positive rate was 3.48% among asymptomatic children in kindergartens and primary schools in Beijing Municipality in June 2021, the most common genotype was GII.4 Sydney, and no acute gastroenteritis outbreak was reported over the study period.

#### What are the implications for public health practice?

The asymptomatic norovirus infection was relatively low among kindergarten children and primary school students in summer. Norovirus genotypes in asymptomatic children were similar to those circulating in the symptomatic cases. Asymptomatic norovirus infection may play a limited role in causing acute gastroenteritis outbreaks.

Norovirus is the dominant causative agent of acute gastroenteritis (AGE) throughout the world, and both symptomatic and asymptomatic persons may be the source of infection (1–3). Asymptomatic persons have no obvious clinical symptoms and are easily neglected. Children in kindergartens and primary schools are the high-incidence groups of AGE due to norovirus (1–2), but asymptomatic infection among them is seldom reported. In this study, norovirus was detected among 460 healthy children, with a positive rate of 3.48%, and GII.4 Sydney was the dominant genotype, the same as in children with AGE. The results suggested that noroviruses circulating in asymptomatic children had a common source with those in AGE cases. However, there were no acute gastroenteritis outbreaks

in schools or kindergartens 4 weeks before and after the sampling, suggesting that asymptomatic children may have a limited role in causing AGE outbreaks.

This is a cross-sectional study in Fengtai District in Beijing in June 2021, and children aged 3–9 years without symptoms of AGE within 4 weeks were recruited in kindergartens and primary schools. According to the economic development levels and geographical characteristics, three communities were selected, including developed FZ (Fangzhuang) community in the east, moderately developed FT (Fengtai) community in the middle, and less developed WZ (Wangzuo) community in the west. A total of 3 kindergartens and 2 primary schools were selected from each community; 10 children were selected from each of the senior, middle, and junior classes in each kindergarten; and 10 students were selected from each of the 1st, 2nd, and 3rd grades in each primary school. Noroviruses were detected using the real-time RT-PCR detection kit (XABT, Beijing, China). Following the kit instructions, if the Ct value  $\leq 35$ , or the Ct value of two PCR tests is between 35–40, the result is judged positive. Semi-nested RT-PCR assays for partial VP1 gene of the norovirus genome were performed, using COG1F/G1SKR and G1SKF/G1SKR for norovirus GI, COG2F/G2SKR and G2SKF/G2SKR for norovirus GII, yielding 330 bp and 344 bp PCR products, respectively (2,4–5). The PCR products were sequenced directly or by cloning sequencing, and the sequences were genotyped using Norovirus Typing Tool Version 2.0 (<https://www.rivm.nl/mpf/typingtool/norovirus>). The phylogenetic trees were constructed using the maximum likelihood method through MEGA software (version 6.0, Mega Limited, Auckland, New Zealand). The initial data was collated and imported into WPS Spreadsheets 2016 (Kingsoft Inc., Beijing, CHN), and statistical analyses were done using SPSS 19.0 software (SPSS, Chicago, IL, USA). Chi-square test was used to compare the positive rates,

and  $P < 0.05$  was considered statistically significant.

In this study, a total of 460 stool specimens were collected, and the positive rate of norovirus was 3.48% (16/460). There was no significant difference in the positive rates among children of different genders, institutions, and communities (Table 1).

In this study, the norovirus positive rate was 8.33% (6/72) in children aged 6 to 7 years, followed by 6.25% (4/64) aged 7 to 8 years, 3.33% (4/120) aged 3 to 4 years, and 2.41% (2/83) aged 4 to 5 years. No norovirus was detected in children aged 5 to 6 years (0/82) and aged 8 to 9 years (0/39).

Of 16 positive stool specimens, 5 were positive for GI, with a median Ct value of 32.6 (range 26.32–37.05); 10 were positive for GII, with a median Ct value of 35.12 (range 24.01–38.23); and 1 was mixed infection of GI (Ct value 33.9) and GII (Ct value 36.5). Of the GI positive stool specimens, 4 GI strains were sequenced successfully, 3 strains were identified as GI.3d and 1 strain was GI.5a (GenBank accession numbers: ON197358–ON197361). GI.3d strains had the highest identities (99.3%–99.6%) with reference strains MW255382/Hu/2020/Chengdu/CHN, MN922742/Hu/2019/Taiwan/CHN and MZ022026/Hu/2020/KRChengdu/CHN/2020, MN922742/Taiwan/CHN/. GI.5a strains had the same identities as reference strains MK121731/Hu/2018/Sichuan/CHN, OK562729/Hu/2021/Guangzhou/CHN, LC544080/Hu/2018/MY (Figure 1). Of 11 GII positive stool specimens, 9 were sequenced successfully, 4 were infected with single genotypes (1 GII.2, 1 GII.6a and 2 GII.4 Sydney), 5 were infected with mixed genotypes (2 GII.2 and GII.4 Sydney, 1 GII.2 and GII.17, 2 GII.6a and GII.4 Sydney). The GenBank accession numbers of 14 GII strains were

ON197537–ON197550. GII.4 Sydney were most common (6/14), which had the highest identities (99.2%–100%) with reference strains OL336382/Hu/2019/Beijing/CHN, OL336366/Hu/2021/Beijing/CHN, MW205658/Hu/2019/Beijing/CHN, OM368736/Hu/2019/Beijing/CHN, MW205667/Hu/2020/Beijing/CHN, MW205571/Hu/2019/Beijing/CHN (Figure 2).

This study found 4 cluster asymptomatic infections in the 4 classes in primary schools, of which 3 were caused by GII noroviruses and 1 was caused by GI noroviruses, and 2 or 3 children were involved in each incident. No norovirus related outbreaks were reported in the asymptomatic infection children's classes 4 weeks after sampling.

## DISCUSSION

In this study, the prevalence of asymptomatic norovirus infection in summer was 3.48% in Beijing Municipality, and reports of norovirus outbreaks in Beijing were fewer during the summer (2). The positive rate of norovirus among outpatient children with diarrhea in summer was far lower than that in the whole year (9.5% vs. 16.5%) in Beijing (4). Cheon et al. also found that the prevalence of norovirus in asymptomatic children aged 4 to 6 in summer was lower than that in winter (2.9% vs. 5.5%) in the Republic of Korea (6). Asymptomatic norovirus infections were observed in 11.7% (19/163) and 13.1% (12/90) in Nicaragua and Brazil (7–8), significantly higher than that in this study. The reason may be that their enrolled children were under 5 years old, and most asymptomatic children were under 2 years old (7–8), the age group with a higher incidence

TABLE 1. Epidemiological characteristics of asymptomatic norovirus infection in children.

Group	Total number	Number of positives	Positive rates (%)	$\chi^2$	P value
Gender					
Male	219	7	3.20	0.099	0.753
Femal	241	9	3.73		
Institution				3.072	0.08
Primary schools	190	10	5.26		
Kindergartens	270	6	2.22		
Community				1.692	0.429
FZ	160	8	5.00		
FT	150	4	2.67		
WZ	150	4	2.67		

Abbreviation: FZ=Fangzhuang, FT=Fengtai, WZ=Wangzuo.

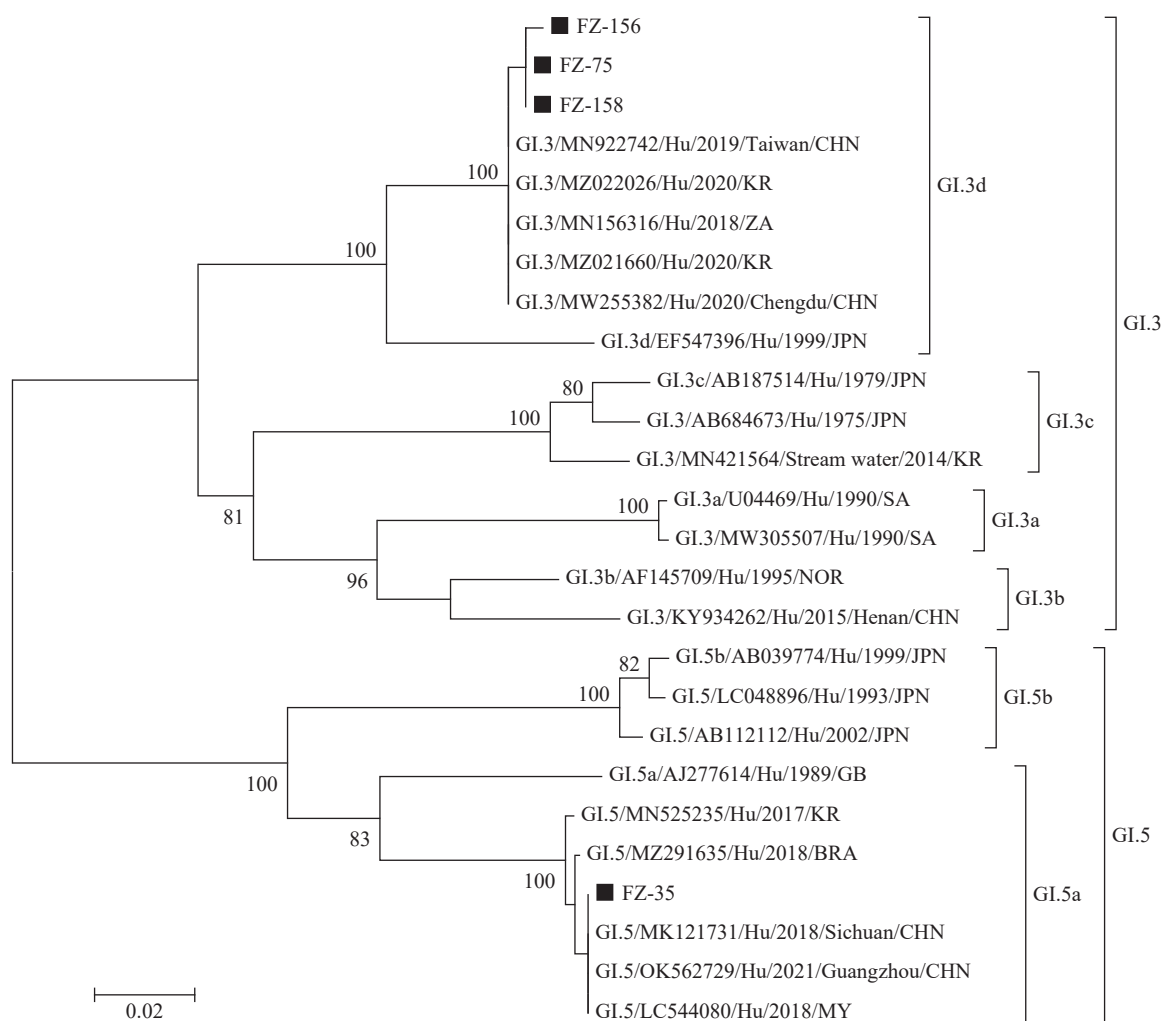


FIGURE 1. Phylogenetic analysis of GI noroviruses based on partial VP1 gene (291 bp).

Note: Norovirus strains detected in this study were marked with black squares. The reference sequences were retrieved from GenBank. The trees were generated using the maximum likelihood method with the nucleotide substitution model Kimura 2-parameter + Gamma + Invariant sites. Bootstrap values, estimated from 1,000 replicates, are indicated at each node. The scale bar indicates the number of nucleotide substitutions.

of norovirus infection (4,9).

A higher positive rate of norovirus cases was found in the developed FZ community in this study, and norovirus outbreaks also occurred mainly in economically developed areas in China (1–2). This trend may be caused by the high population density and rapid population mobility in economically developed regions, which facilitate the spread of infectious diseases. The positive rate in primary school was higher than that in kindergarten (5.26% vs. 2.22%), similar to those in Changzhou City, Jiangsu Province (4.70% vs. 2.68%) (10). It could be because the children in primary school have more social activities than those in kindergarten, and more children, both in class and school, have a higher chance of contracting an infection.

This study's reported genotypes were consistent with those in symptomatic children in Beijing, and GII.4 Sydney was the most common genotype (4). This result suggests that asymptomatic and symptomatic infections have a common source.

We found that the Ct values of norovirus in children with asymptomatic infection were higher, and the optimal Ct value cutoff for attributing infectious intestinal disease to norovirus was reported as 31 (11). A recent study also showed that the Ct values of GII norovirus in children with diarrhea were significantly lower than those without diarrhea. GII norovirus was associated with diarrhea when the Ct value was  $\leq 25$  (11). Shioda et al. found that the Ct value of norovirus infection in symptomatic cases ( $25.3 \pm 1.2$ ) was lower than in asymptomatic persons ( $28.5 \pm 1.4$ ) (12). In this

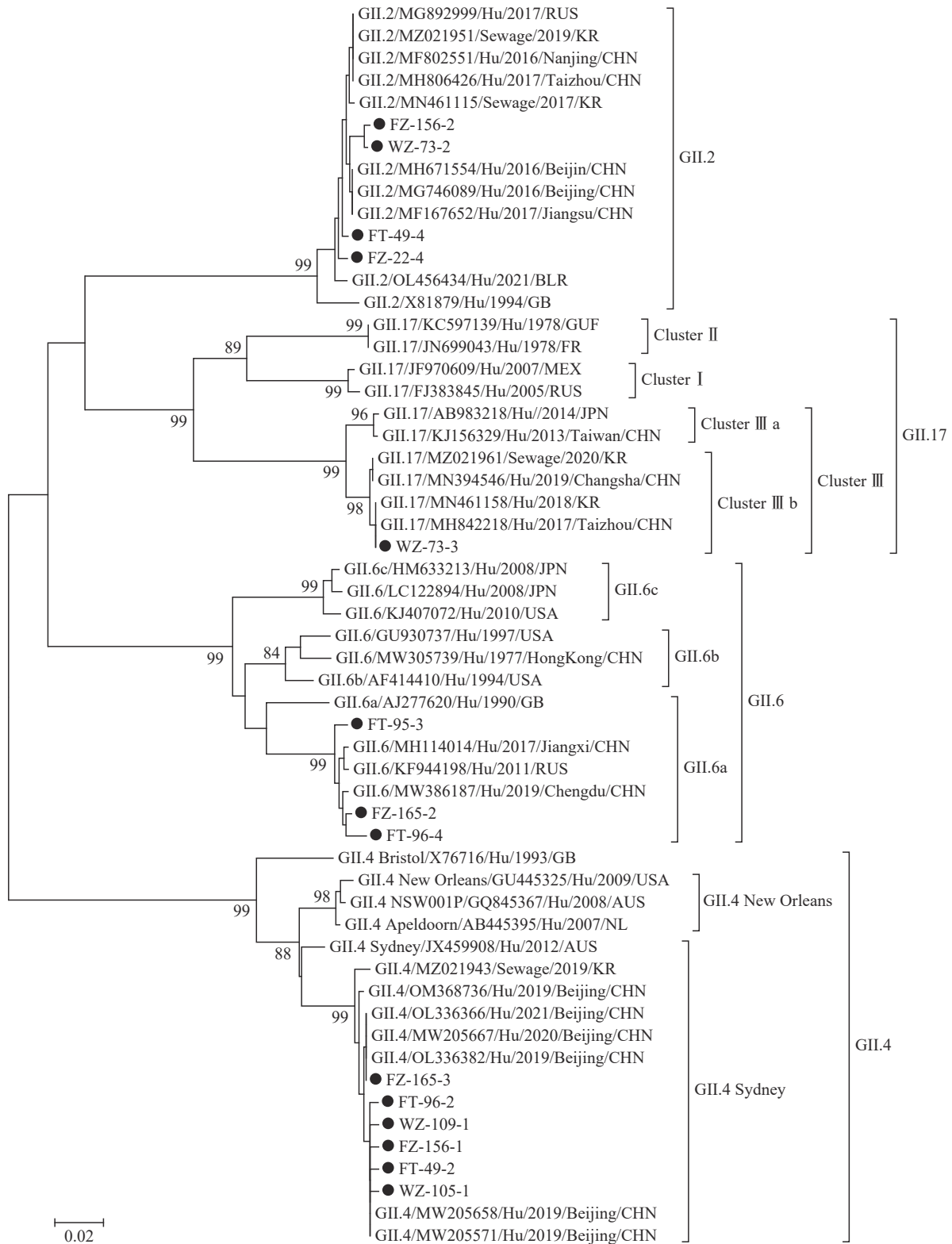


FIGURE 2. Phylogenetic analysis of GII noroviruses based on partial VP1 gene (283 bp). Note: Norovirus strains detected in this research were marked with black dots. The reference sequences were retrieved from GenBank. The trees were generated using the maximum likelihood method with the nucleotide substitution model Kimura 2-parameter + Gamma. Bootstrap values, estimated from 1,000 replicates, are indicated at each node. The scale bar indicates the number of nucleotide substitutions.

study, the median Ct value of GI was 32.6, and GII was 35.1. This is consistent with the results of Kobayashi et al. for Ct values in asymptomatic adults, GI (34.4) and GII (35.4) (13). These results prove that asymptomatic norovirus infections have higher Ct values.

In this study, 4 clustered asymptomatic infections were found, but no norovirus outbreak was reported 4 weeks before and after sampling. This finding suggested that asymptomatic infection was not easy to cause the outbreak. The possible reasons are the low viral load and the high temperature in summer, which lead to the decline in virus survival and transmission. Benjarat et al. also found that asymptomatic people were more likely to cause family clustered infections in winter than in summer and that most were asymptomatic clustered infections (3).

This study was subject to some limitations. First, this is a cross-sectional study in one month, not throughout the year. Second, the source of asymptomatic infections was not investigated, for example, whether it came from infected family members.

The norovirus-positive rate was 3.48% among asymptomatic children in kindergartens and primary schools in summer, and norovirus genotypes in asymptomatic children were similar to those circulating in the symptomatic cases. Asymptomatic infected children present a lower viral load and may have a limited role in causing AGE outbreaks.

**Funding:** Supported by the 2019 Fengtai District Health System Scientific Research Project (No. 2019-128); Fengze program support: Fengtai District Applied Public Health Advanced Talent Development Program (2021-40558).

doi: 10.46234/ccdcw2023.031

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Submitted: January 30, 2023; Accepted: February 20, 2023

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