



BRIEF REPORT

Occurrence of Norovirus GII.4 Sydney Variant-related Outbreaks in Korea

Sunyoung Jung^a, Bo-Mi Hwang^a, Hyun Ju Jeong^a, Gyung Tae Chung^a,
Cheon-Kwon Yoo^a, Yeon-Ho Kang^b, Deog-Yong Lee^{a,*}

^aDivision of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health, KCDC, Osong, Korea.

^bDivision of Bio-Safety Evaluation and Control, Korea National Institute of Health, KCDC, Osong, Korea.

Received: February 5,
2015
Revised: October 2,
2015
Accepted: October 2,
2015

KEYWORDS:

GII.4 Sydney variant,
norovirus,
outbreak

Abstract

Human noroviruses are major causative agents of food and waterborne outbreaks of nonbacterial acute gastroenteritis. In this study, we report the epidemiological features of three outbreak cases of norovirus in Korea, and we describe the clinical symptoms and distribution of the causative genotypes. The incidence rates of the three outbreaks were 16.24% (326/2,007), 4.1% (27/656), and 16.8% (36/214), respectively. The patients in these three outbreaks were affected by acute gastroenteritis. These schools were provided unheated food from the same manufacturing company. Two genotypes (GII.3 and GII.4) of the norovirus were detected in these cases. Among them, major causative strains of GII.4 (Hu-jeju-47-2007KR-like) were identified in patients, food handlers, and groundwater from the manufacturing company of the unheated food. In the GII.4 (Hu-jeju-47-2007KR-like) strain of the norovirus, the nucleotide sequences were identical and identified as the GII.4 Sydney variant. Our data suggests that the combined epidemiological and laboratory results were closely related, and the causative pathogen was the GII.4 Sydney variant strain from contaminated groundwater.

Norovirus is a major cause of epidemic acute nonbacterial gastroenteritis in humans worldwide. Norovirus infection is common in all age groups and is characterized by a low infection dose and efficient transmission with typical fecal-oral routes, as well as airborne spread and environmental contamination [1–3]. Human norovirus GII.4 is prominent in all genogroups and genotypes, and GII.4 variants were divided into 13

subcluster types along with epidemic year and genetic characterization. In particular, the GII.4 Sydney strain, named as 2012 variant, is relatively new and predicted as the next prominent strain [4–6]. There are several reports on GII.4-associated gastroenteritis, but there is a lack of environmental and molecular–epidemiological data. In Korea, three outbreaks associated with norovirus GII.4 Sydney variant occurred in a middle and high school

*Corresponding author.

E-mail: leedy0610@korea.kr (D.-Y. Lee).

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License (<http://creativecommons.org/licenses/by-nc-nd/4.0>) which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

E-mail: leedy0610@korea.kr (D.-Y. Lee).

Table 1. Comparison of epidemiological and laboratory characteristics.

Features of outbreaks	Outbreak A	Outbreak B	Outbreak C
Epidemiological features			
Attack rate (%)	326/2007 (16.24)	27/656 (4.1)	36/214 (16.8)
Symptomatic patients	326	27	36
Date of onset	Nov 21, 2012	Nov 30, 2012	Nov 30, 2012
Scale(s)	Large	Medium	Medium
Type of setting	School	School	School
Clinical symptoms (%)			
Diarrhea	326/326 (100)	23/27 (85.2)	26/36 (72.2)
Nausea	223/326 (68.4)	24/27 (88.9)	24/36 (66.7)
Abdominal pain	205/326 (62.9)	25/27 (92.6)	17/36 (47.2)
Vomiting	156/326 (47.9)	14/27 (51.9)	26/36 (72.2)
Fever	190/326 (58.3)	14/27 (51.9)	17/36 (47.2)
Laboratory test			
No. of examinations of patients	99	10	22
Positive samples	46	2	12
Genogroup I	—	—	—
Genogroup II	46	2	12
No. of examinations of food-handlers	28	14	5
Positive samples	2	5	2
Genogroup I	—	—	—
Genogroup II	2	5	2
Environmental samples	13		
Causative foods (unheated foods)	9		
Genogroup I	—		
Genogroup II	9		
Supplemental water	3		
Genogroup I	—		
Genogroup II	3		
Groundwater	1		
Genogroup I	—		
Genogroup II	1		

setting in different cities on November 21st–30th, 2011. In this study, we describe the investigation of three outbreaks caused by the GII.4 Sydney variant, which was traced to contaminated groundwater from a supply manufacturing company.

In November 2011, three outbreaks of acute gastroenteritis occurred in three different regions. An

epidemiological study was performed with a retrospective cohort study and cases were defined as patients who presented with diarrhea, nausea, abdominal pain, vomiting, and fever. Attack rates were 16.27% (326/2,007) in Outbreak A, 4.1% (27/656) in Outbreak B, and 16.8% (36/214) in Outbreak C. The main symptoms were diarrhea, nausea, abdominal pain, vomiting, and fever

Table 2. Oligo nucleotide sequences of primers used in this study.

Genogroup	Name of primer	Sequence (5' → 3')	Application
GI	NoGI-F1	ATGGCCATGTTCCGITGGATG	Stool specimen
	NoGI-F2	CGGGCCCCGAATTYGTAAATGATG	
	NoGI-R	CCAACCCARCCATTRTACATYTG	Stool & environmental samples
	GI-F1M	CTGCCCGAATTYGTAAATGATGAT	
	GI-F2	ATGATGATGGCGTCTAAGGACGC	
	NoGI-R	CCAACCCARCCATTRTACATYTG	
GII	NoGII-F1	CCCTCGAGGGCGATCGCAATCT	Stool specimen
	NoGII-F2	CACAATTGTGAATGAAGATGGCGTCGA	
	NoGII-R	CCRCCIGCATRICRTRTACAT	Stool & environmental samples
	GII-F1M	GGGAGGGCGATCGCAATCT	
	GII-F3M	TTGTGAATGAAGATGGCGTCGART	
	NoGII-R	CCRCCIGCATRICRTRTACAT	

(Table 1). Patients from Outbreak A had mainly diarrheal symptoms (100%), patients from Outbreak B had abdominal pains (92.6%), and patients from Outbreak C had diarrhea (72.2%) and vomiting (72.2%). According to epidemiological findings, three outbreaks occurred in different regions, but the same food manufacturers supplied the three regions with unheated food around the same time. This food manufacturer had used groundwater for the preparation of unheated food.

A laboratory test was performed on 131 fecal specimens of cases, 47 from food handlers, and 13 environmental samples. For virus detection, viral RNA was extracted using a commercialized RNA preparation kit (GM-AUTOPREP Kit, Green Mate Biotech Crop, Seoul, Korea). The norovirus was screened using commercialized real-time reverse transcription polymerase chain reaction (RT-PCR) (Bioneer, Daejeon, Korea) according to the manufacturer's manual. From 131 fecal specimens from patients, 60 patients (45.8%) were determined as norovirus-positive with real-time RT-PCR. In each case, 46.5% (46/99) were norovirus-positive in Outbreak A, 20% (2/10) in Outbreak B, and 54.5% (12/22) in Outbreak C. Nine food handlers (19.1%) were determined as norovirus-positive from the 47 food handlers tested. All environmental samples tested were proven as norovirus-positive using real-time RT-PCR. Environmental samples included causative unheated food, supplemental water to prepare food in the manufacturer's company, and groundwater used as supplemental water (Table 1).

Amplification of norovirus genes were performed using one-step RT-PCR with norovirus specific primers listed in Table 2. Subsequently, seminested PCR was performed to partially amplify the norovirus *capsid* gene. Environmental samples were tested according to the methods of the Ministry of Food and Drug Safety and stool specimens were tested according to the methods of the Korea National Institute of Health. Amplified PCR products were purified using Millipore plate MSNU030 (Millipore SAS, Molsheim, France) and were sequenced with the Big Dye terminator version 3.1 sequencing kit and a 3730xl automated sequencer (Applied Bio systems, Foster City, CA, USA). Nucleic acid sequences were aligned with information of genotype sequence using MegAlign package (Window version 3.12e, DNASTAR, Madison, WI) in the DNASTAR program, and the phylogenetic tree was generated using the neighbor-joining method [7,8]. Most genotypes were determined as GII.4 (Hujju-like) strains detected in patients, food handler fecal samples, and environmental samples. However, GII.3 was only detected in one sample collected from unheated food (Figure 1). To compare sequence identity, each group showed a similarity of 96.8–99.5% (Outbreaks A and B), 94.7–100% (Outbreaks A and C), 96.3–100% (Outbreak A and environmental samples),

Table 3. Distribution of norovirus GII-4 variant analyzed in this study.

Name of variant	Outbreak A	Outbreak B	Outbreak C	Environmental samples
2006b	—	—	1 (7.1)	—
2010	—	—	—	2 (16.7)
2012	44 (100)	7 (100)	13 (92.9)	10 (83.3)

Data are presented as *n* (%).

89.9.3–99.5% (Outbreaks B and C), 95.2–100% (Outbreak B and environmental samples), and 94.7–100% (Outbreak C and environmental samples; data not shown).

GII.4 variant analysis was performed using NoroNet alignment tools (<http://noronet.nl>) [4–6]. Prominent strains were 2012 Sydney variants in these three outbreaks, although 2006b and 2010 New Orleans were also detected. Analysis of strains from Outbreaks A and B detected the GII-4 Sydney strain but a 2006b variant was detected in Outbreak C. In the environmental samples, the 2010 New Orleans strain was detected in two unheated food samples. However, the 2012 Sydney strain occupied all environmental samples (Table 3).

Three outbreaks occurred with the same norovirus GII.4 Sydney variant in different cities at the same time and showed typical symptoms of norovirus-related acute gastroenteritis. These outbreaks were associated with unheated food which was commonly provided from the same manufacturer. However, there were several environmental factors that led to infection, such as food handlers, unheated food, supplemental water, and groundwater. GII.4 strains were already well-known to be prominent in humans and the current prominent Sydney variant has been well-reported in outbreak cases [4–6,9–11]. However, there is a lack of reports on 2012 Sydney variant-associated cases. Our data were fully analyzed based on clinical, environmental, and molecular analysis. In conclusion, this study revealed three outbreaks of acute gastroenteritis caused by norovirus GII that came from contaminated groundwater, and the major causative pathogen was the GII-4 Sydney variant.

Conflicts of interest

The authors have nothing to declare.

Acknowledgments

This study was supported by the National Norovirus Surveillance System (4851-304-210) and conducted by the Korea National Institute Health and Seoul

and Gyungbuk Institute of Health and Environment Research.

References

1. Rockx B, De Wit M, Vennema H, et al. Natural history of human calicivirus infection: a prospective cohort study. *Clin Infect Dis* 2002 Aug;35(3):246–53.
2. Maunula L, Miettinen IT, von Bonsdorff CH. Norovirus outbreaks from drinking water. *Emerg Infect Dis* 2005 Nov;11(11):1716–21.
3. Hewitt J, Bell D, Simmons GC, et al. Gastroenteritis outbreak caused by waterborne norovirus at a New Zealand ski resort. *Appl Environ Microbiol* 2007 Dec;73(24):7853–7.
4. Belliot G, Kamel AH, Estienney M, et al. Evidence of emergence of new GGII.4 norovirus variants from gastroenteritis outbreak survey in France during the 2007-to-2008 and 2008-to-2009 winter seasons. *J Clin Microbiol* 2010 Mar;48(3):994–8.
5. Vega E, Barclay L, Gregoricus N, et al. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. *Emerg Infect Dis* 2011 Aug;17(8):1389–95.
6. van Beek J, Ambert-Balay K, Botteldoorn N, et al. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. *Euro Surveill* 2013 Jan;18(1):8–9.
7. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985 Jul;39(4):783–91.
8. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987 Jul;4(4):406–25.
9. Fonager J, Hindbæk LS, Fischer TK. Rapid emergence and antigenic diversification of the norovirus 2012 Sydney variant in Denmark, October to December, 2012. *Euro Surveill* 2013 Feb;18(9):1–4.
10. Polkowska A, Ronnqvist M, Lepisto O, et al. Outbreak of gastroenteritis caused by norovirus GII.4 Sydney variant after a wedding reception at a resort/activity centre, Finland, August 2012. *Epidemiol Infect* 2014 Sep;142(9):1877–83.
11. Wong TH, Dearlove BL, Hedge J, et al. Whole genome sequencing and *de novo* assembly identifies Sydney-like variant noroviruses and recombinants during the winter 2012/2013 outbreak in England. *Virology* 2013 Nov;10:335.