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Emergence of GII.4 Sydney Norovirus in South Korea During the Winter of 2012–2013

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Norovirus is the major cause of acute gastroenteritis worldwide. Between November 2012 and June 2013, 1718 stool samples were requested for norovirus antigen testing in the metropolitan areas of South Korea, and 91 samples were genotyped. The norovirus antigen-positive rate peaked at 52.8% in December 2013. A novel norovirus GII.4 variant, GII.4 Sydney 2012, was the most frequently found genotype (60.4%) during this period. This study demonstrates that norovirus activity increased during the winter of 2012–2013 in South Korea and that norovirus GII.4 Sydney 2012 was the cause of the norovirus epidemic during this period.

Keywords: Norovirus, genotype, GII.4 Sydney

Noroviruses are the major cause of acute epidemic and sporadic gastroenteritis in all age groups worldwide [6, 9, 15]. Norovirus is an RNA virus of the *Caliciviridae* family and is classified into five genogroups (GI–GV) according to the polymerase and capsid protein sequences. Thus far, 8 GI and 23 GII capsid genotypes have been described [6]. Continuous monitoring of norovirus molecular epidemiology is necessary for preventing and controlling the spread of norovirus outbreaks. Norovirus genotype GII.4, which evolves rapidly over time, has been associated with most norovirus outbreaks. New GII.4 variants emerge every 2–3 years and become the cause of global epidemics of acute gastroenteritis. These GII.4 variants seem to have higher epidemiological fitness than that of other genotypes. Since 1995, at least five GII.4 variants have been responsible for global epidemics: US 1995/96 in the late 1990s, Farmington Hills virus in 2002, Hunter virus in 2004, Den Haag 2006b virus in 2007–2008, and New Orleans virus 2009 in 2009–2012 [2, 11]. Other GII.4 variants that have been associated with localized epidemics in specific regions but not with global epidemics include Japan 2001, Henry 2001, Asia 2003, Yerseke 2006a, Osaaka 2007, and Apeldoorn 2008. In March 2012, a new GII.4 norovirus variant designated GII.4 Sydney 2012 was identified in Australia [16]. This new strain was reported in New Zealand, Japan, Hong Kong, South Africa, Russia, Western Europe, Canada, and the USA [1, 4, 17]. In South Korea, norovirus infection is most prevalent during autumn and winter [13]. GII.1 was the most frequently detected genotype in Gyeonggi from 2001 to 2005 [7], whereas GII.4 was most frequent in Seoul from 2004 to 2007 [14] and 2008 to 2010 [3]. We investigated the molecular epidemiology of the norovirus outbreak during the winter of 2012–2013 in the metropolitan areas of South Korea.

This study was approved by the Institutional Review Board of Hallym University Dongtan Sacred Heart Hospital (IRB No. 2013-030). A total of 1,718 stool samples were requested for norovirus antigen testing from four university hospitals (2 in Seoul and 2 in Gyeonggi Province) in the metropolitan areas of South Korea between November 2012 and June 2013. Of these, 254 samples (14.8%) showed positive results using the RIDASCREEN Norovirus antigen test kit (R-Biopharm, Germany) [12]. From January 2013, we started to collect norovirus antigen-positive stool samples for norovirus genotyping study. Stool samples were diluted to a 10% stool suspension in phosphate-buffered saline and stored at −70°C until use for norovirus PCR and norovirus genotyping. From January to June 2013, 151 norovirus antigen-positive samples were obtained; 25 samples could not be analyzed because of insufficient stool

Table 1. The results of positive norovirus antigen tests from November 2012 to June 2013.

	No. of requested	No. of positive results	Positive rate
Nov. 2012	93	36	38.7%
Dec. 2012	127	67	52.8%
Jan. 2013	225	53	23.6%
Feb. 2013	300	38	12.7%
Mar. 2013	252	27	10.7%
Apr. 2013	256	16	6.3%
May 2013	247	11	4.5%
June 2013	218	6	2.8%
Total	1718	254	14.8%

volume for additional PCR and genotyping. Thus, 126 samples were analyzed. These 126 norovirus antigen-positive samples were screened for norovirus RNA using the Seeplex DV mRT-PCR assay (Seegene, South Korea) [5] and 97 samples were positive by PCR. In 6 of the 97 PCR-positive samples, we failed to obtain genotyping results. Therefore, 91 samples were successfully genotyped between January 2013 and June 2013. Of 91 norovirus-positive samples, 45 (49.5%) were collected from males, and the overall median age of the donors was 1.7 years (range, 1 week to 74 years). Seventy samples (76.9%) were from patients aged less than 5 years.

Norovirus genotyping was performed as described previously [8]. For 1-step RT-PCR, the specific primers GI-F1M (nt 5,342)/GI-R1M (nt 5,671) and GII-F1M (nt 5,058)/GII-R1M (nt 5,401), which target open reading frame 2 (ORF2)-encoding capsid protein (VP1), were used. Nested PCR was also performed using the primers GI-F2 (nt 5,357)/GI-R1M (nt 5,671) and GII-F3 (nt 5,088)/GII-R1M (nt 5,401). The products from the nested PCR were visualized by agarose gel electrophoresis and analyzed by DNA sequencing. The nucleotide sequences were analyzed using ABI Prism BigDye Terminator version 3.1 (Applied Biosystems, USA), and an automated genotyping tool was used to identify norovirus genotypes (available at http://www.rivm.nl/mpf/norovirus/typingtool) [10].

The rate of positive norovirus antigen tests between November 2012 and June 2013 is shown by month in Table 1. The peak incidence was found in December 2012, when 52.8% of requested samples were norovirus antigen-positive. Subsequently, the positive rate gradually decreased to 2.8% in June 2013. Ninety-one samples were genotyped between January 2013 and June 2013 (Table 2). Eighty nine GII genotypes (97.8%) and 2 GI genotypes (2.2%) were detected during this period. GII.4 (63.7%) was the most frequent genotype and GII.17 (6.6%), GII.6 (5.5%), GII.13 (5.5%), GII13/14 (4.4%), GII.2 (4.4%), GII.3 (3.3%), GII.21 (3.3%), GI.4 (1.1%), and GI.9 (1.1%) were also detected. The GII.21 genotype has not been detected previously in Korea. A novel variant, GII.4 Sydney 2012, which has been previously reported in Australia, New Zealand, Japan, Western Europe, Canada, and the USA, was the predominant genotype during this period, comprising 55/58 NoV GII.4-positive

Table 2. The distribution of norovirus genotypes identified.

Genotype	No. of positive samples	Positive rate
GI		
GI.4	1	1.1%
GI.9	1	1.1%
GII		
GII.2	4	4.4%
GII.3	3	3.3%
GII.4	58	63.7%
GII.4 Sydney	55	60.4%
GII.4 Non-Sydney (Den Haag 2006b)	3	3.3%
GII.6	5	5.5%
GII.8	1	1.1%
GII.13	5	5.5%
GII.13/14	4	4.4%
GII.17	6	6.6%
GII.21	3	3.3%
Total	91	100%

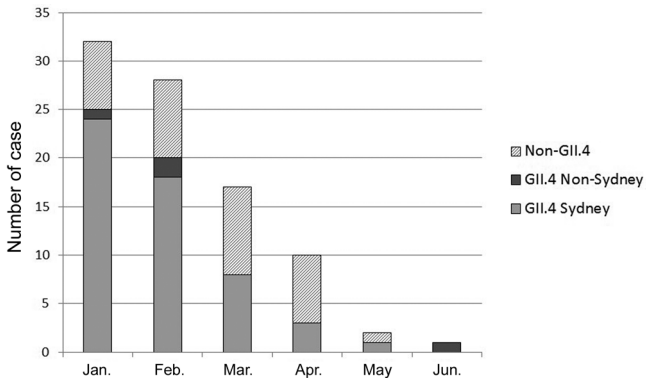

Fig. 1. Monthly distribution of norovirus GII.4 variants in 2013.

samples (94.8%) (Fig. 1 and Table 2). The remaining three samples were of the NoV GII.4 Den Haag 2006b genotype.

This study had some limitations. First, the data collected may not be representative of the overall molecular epidemiological trends in South Korea. Second, the norovirus antigen-positive rate in the winter of 2012–2013 may be exaggerated, considering only 97 samples of 126 norovirus antigen-positive samples showed PCR-positive results. Nonetheless, these data are indicative of a norovirus outbreak in the winter of 2012–2013 and the distribution of norovirus genotypes.

In conclusion, the present study demonstrated that norovirus activity increased during the winter of 2012–2013 in South Korea and that this increased activity was related to the emergence of a new NoV GII.4 variant designated GII.4 Sydney 2012, which was similar to global trends.

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