Temporal Genotype Variation of Norovirus Genogroup II in Oysters Analyzed by Pyrosequencing

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1. Introduction

A significant number of norovirus outbreaks have occurred worldwide since the mid-1990s, with the genogroup II genotype 4 (GII.4) as the major cause. The novel GII variant, GII.17 Kawasaki 2014, emerged in the 2014/2015 norovirus season and replaced the previously prevalent GII.4 Sydney 2012 variant. Oyster can accumulate norovirus when grown in contaminated marine environment and are considered one of the most important pathways of norovirus transmission. For better understanding of the norovirus genotypes circulating in the human population and those released in the environment in the 2014−2015 norovirus season, we investigated temporal variation of norovirus genogroup II in oysters by using pyrosequencing techniques in this study.

2. Methodology

Oyster samples (3 individuals per station, 2 stations: St. A and St. B) were collected weekly from November 5th, 2014 to March 26th, 2015, in Miyagi Prefecture, Japan. Digestive tissues were excised from the oyster samples and mixed with an enzyme solution containing amylase (6.24 mg/mL), proteinase-K (0.25 mg/mL), and lipase (6.24g/mL) for virus extraction. In brief, the virus extraction was performed with two stainless beads on Micro Smash (TOMY, Tokyo, Japan) at 4,200 rpm for 60 s and then incubated with the enzyme solution for 60 min at 37°C followed by 15 min at 60°C. Viral RNA was extracted using the NucliSENS miniMAG Kit following the manufacturer's instructions. Complementary DNA was reverse transcribed and then applied to CFX96 Touch Real-Time PCR detection system (Bio-Rad) for quantification of NoV GII. Only positive samples selected by qPCR assays were carried on to the pyrosequencing steps.

For positive samples screened by RT-qPCR, the cDNA was used for a nested PCR assay using the COG2F/G2SKR and G2SKF/G2SKR primer sets to amplify the RdRp and capsid N/S-encoding domain (RdRp-N/S) region. The nested PCR products were separated by agarose gel electrophoresis and products with the expected length (344 bp) were excised from the gel and purified using the Qiagen Gel Extraction Kit (Qiagen). The purified products were applied in a fusion PCR assay, and subjected to pyrosequencing using the GS Junior system (Roche Applied Science) and bioinformatics analysis was performed as described by Kazama *et al.* (2016).

3. Results and discussion

The concentration of NoV GII genomes was determined by RT-qPCR assays. All 18 samples were positive for GII in oyster samples. Three predominant genotypes (GII.3, GII.17, and GII.4) were found in oyster samples, detected in 100%, 52.95% and 29.4% of the samples, respectively. GII.17, a genotype previously undetected in oysters, was identified in our oyster samples for the first time (Fig 1). In the 2014−2015 norovirus season, the GII.17 Kawasaki 2014 variant was detected more frequently than the GII.4 Sydney 2012 variant in gastroenteritis cases in our study area, Miyagi Prefecture, as reported in other areas in Japan. Genetically closely related to Kawasaki 2014 identified in oysters taken from January to March of 2015, forming a distinct subclade of the Kawasaki 2014 GII.17 variant, as shown in Fig 2. The simultaneous existence and dominance of the GII.17 strains in sewage, oysters, and gastroenteritis cases highlights the importance of studying diverse norovirus genotypes circulating in the human population and those released in the environment.

The emergence and spread of the novel GII.17 variant could represent a challenge for norovirus vaccination, which target the globally predominant GII.4 norovirus. Multiple viral genes existing in oysters could generate new recombinant viruses, bringing additional risks to human beings. Continuous monitoring should be done in the future on norovirus behaviors in oysters and water environment, as well as epidemiology, for better understanding the evolution of norovirus and reducing the risk of public health.

Acknowledgment

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References

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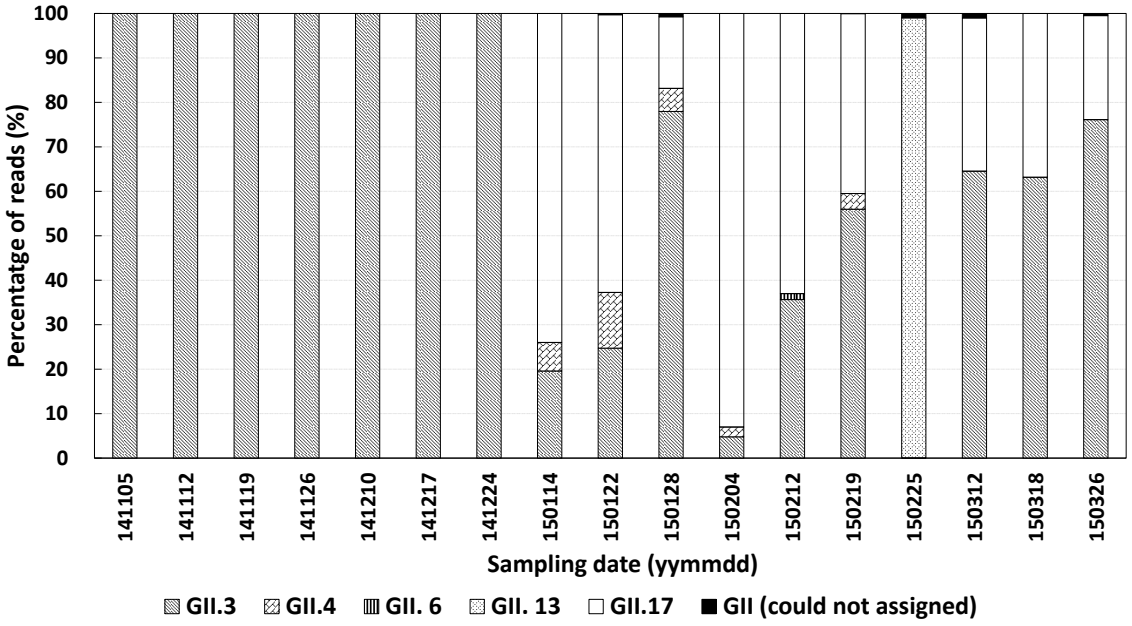

Fig 1 Genotypes detected in weekly oyster samples.

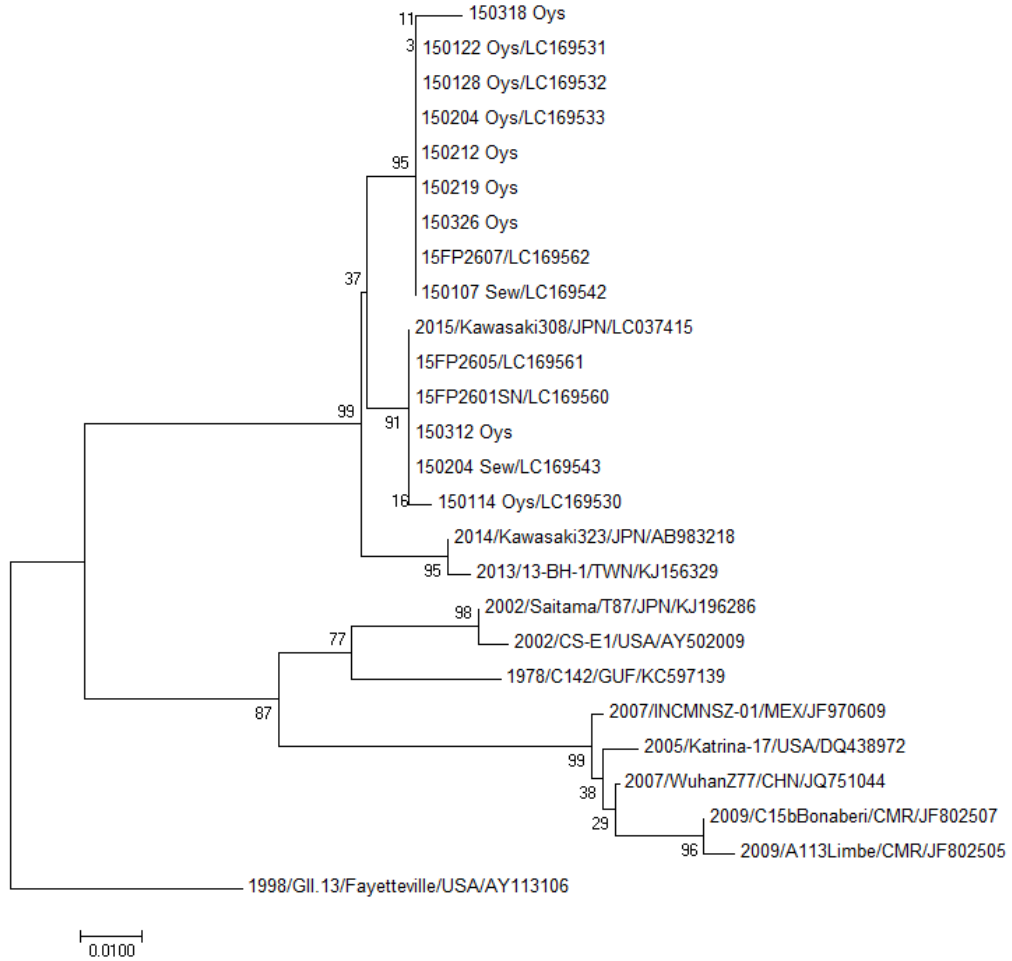

Fig 2 Phylogenetic tree of the RdRp-N/S region of GII.17 strains obtained from oyster samples. Trees were built with the maximum-likelihood method, and bootstrapped with 1,000 repetitions. The obtained sequences are designated with names starting from sampling data (yymmdd) followed by “Oys” for oyster, and accession number. The reference sequences are designated with names following the order of starting year, strain name, country, and accession number.