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Detection and Phylogenetic Analysis of Norovirus in *Corbicula fluminea* in a Freshwater River in Japan

Mika Saitoh[1,2], Hirokazu Kimura[1], Kunihisa Kozawa[1], Osamu Nishio[3], and Akira Shoji*[,2]

[1]*Gunma Prefectural Institute of Public Health and Environmental Sciences, Maebashi, Gunma 371–0052, Japan,* [2]*Department of Biological Sciences, Faculty of Engineering, Gunma University, Kiryu, Gunma 376–8515, Japan, and* [3]*National Institute of Infectious Diseases, Musashimurayama, Tokyo 208–0011, Japan*

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***Abstract*: To study the molecular epidemiology of noroviruses (NoVs) in bivalves residing in freshwater rivers, we detected, quantified and phylogenetically analyzed the NoV genome in purified concentrates obtained from the gills and digestive diverticula of *Corbicula fluminea* in a freshwater river in Gunma Prefecture, Japan. We detected the NoV genome in 35 of the 58 *C. fluminea* samples. Based on our phylogenetic analysis, the NoV genome detected in the samples was classified into 4 genotypes (GI/1, GI/2, GI/3 and GI/4) in genogroup I and 5 genotypes (GII/3, GII/4, GII/5, GII/8 and GII/12) in genogroup II. The phylogenetic tree showed wide genetic diversity among the genogroups. In addition, more than 10^4 copies of the NoV genome were detected in 2 of 35 samples. These results suggest that the freshwater bivalve *C. fluminea* is a reservoir for NoVs, similar to seawater bivalves such as oysters.**

Key words: **Phylogenetic analysis, Norovirus, *Corbicula fluminea*, Freshwater**

Norovirus (NoV) belongs to the family *Caliciviridae* and is a causative agent of acute gastroenteritis in humans. NoV has been the main cause of human food-borne and water-borne non-bacterial acute gastroenteritis (3, 13, 20). In Japan, approximately 45% (12,537 of 28,175 patients) of the total gastroenteritis and food-borne cases in 2004 were attributed to NoV. River water, sewage and seawater have been polluted with the excreta of infected patients, and NoV gastroenteritis outbreaks have been associated with the consumption of bivalves, including oysters (22). Furthermore, viral particles can accumulate in various types of freshwater or seawater bivalves (21, 28). Cooking with inadequate heating or the consumption of raw bivalves has resulted in several instances of NoV food-borne and infectious gastroenteritis in Japan, other Asian countries and France (7, 9, 24). Additionally, person-to-person transmission of NoV occurs more easily than any other mode of transmission. This is because infected patients shed a large amount of NoVs in their feces and/or vomit, while a small NoV load can cause gastroenteritis in humans (as few as 10–100 viral particles can cause infection) (3, 19).

The NoV is classified into two genogroups: genogroup I (GI) and genogroup II (GII). Each group is further subdivided into 14 (GI/1–14) and 17 (GII/1–17) genotypes, respectively (12, 14). Recently, numerous outbreaks due to NoV GII/4 were reported worldwide including in European countries, Japan and the U.S. Data from these outbreaks suggest that the NoV GII/4 detected in patients is related to the NoV GII/4 found in seawater bivalves (7, 25, 27).

In Japan, there are three primary domestic *Corbicula* species: *Corbicula japonica* (*C. japonica*), which is widely distributed in brackish waters, *Corbicula sandai*, which is found in Lake Biwa, and *Corbicula leana*, which lives in freshwater. *C. japonica* has been primarily caught for food. In recent years, however, the fishing of *C. japonica* has been decreasing in Japan, and therefore various corbicula clams are now being imported from foreign countries (10). *Corbicula fluminea* (*C. fluminea*: taiwan shijimi) is variable in its shape, and

Address correspondence to Dr. Akira Shoji, Department of Biological Sciences, Faculty of Engineering, Gunma University, 1–5–1 Tenjincho, Kiryu, Gunma 376–8515, Japan. Fax: +81–277–30–1443. E-mail: shoji@bce.gunma-u.ac.jp

Abbreviations: GI, genogroup I; GII, genogroup II; N-J, neighbor-joining; NoV, norovirus; OTU, operational taxonomic unit; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR.

naturally inhabits mostly freshwater in southeastern Asia, China, Korea and southeastern Russia. *C. fluminea* has been imported into Japan as food, and is now widely distributed in Japanese rivers (Ministry of the Environment, Invasive Alien Species Act (http://www.env.go.jp/index.html); Gulf States Marine Fisheries Commission, *Corbiculla fluminea* (Muller 1977) (http://www.gsmfc.org/)). These corbicula clams might become a source of NoV infection, as seawater bivalves are. In addition, phylogenetic analysis data of the NoV isolated from corbicula clams might be useful to investigate the prevalence of NoV. To examine these issues from a molecular epidemiological approach, we collected *C. fluminea* from river water in Gunma Prefecture in Japan every month in 2004, detected the concentrated NoV, and then analyzed the NoV genomes phylogenetically.

Materials and Methods

Sampling site. The sampling site was a tributary of the Kanna River flowing through Fujioka City in Gunma Prefecture in Japan (Fig. 1). The river stream, including our sampling site, flows along the residential areas of Fujioka City, whose population is approximately 71,000. The river is approximately 2 m wide, and sewage water that is conventionally treated in septic tanks in the houses is discharged into the river.

Samples and preparation of viral suspension. A total of 406 corbicula clams (*C. fluminea*) were collected monthly from January to December 2004, and included 35 clams every month except January, when 21 clams were collected. Since each sample consists of 7 clams, the number of samples was 5 every month except 3 in January. On the day of collection, the clams were shucked and their gills and digestive diverticula were dissected. Subsequently, 1–1.3 g of gills and digestive diverticula (giving a total weight corresponding to that of 7 clams) were homogenized and 10% suspension was prepared with phosphate-buffered saline (PBS) solution without magnesium or calcium. The homogenate was mixed with 0.1 ml of antifoam B (Sigma, St. Louis, Mo., U.S.A.) and homogenized for two 30-sec intervals, then centrifuged with a maximum speed of 18,000 rpm using an Omni-mixer (OCI Instruments, Waterbury, Conn., U.S.A.). Six milliliters of a chloroform:butanol (1:1, vol vol^{-1}) mixture were added to the supernatant of the homogenate (8, 24). The mixture was then homogenized for an additional 30 sec, and 170 ml of Cat-Floc T (Calgon, Elwood, Pa., U.S.A.) was added to the homogenate. The homogenate samples were centrifuged at 3,000 $\times g$ for 30 min at 4 C, and 7 ml of the supernatant was layered on 2 ml of 30% sucrose solution, and ultracentrifuged at 201,000 $\times g$ for 2 hr at 4 C. The pellet was resuspended in 200 µl of DNase/RNase-free water and then stored at −80 C until use.

RNA extraction, RT-PCR and sequencing. Viral RNA was extracted from 138 µl of viral suspension using a QIAamp Viral RNA Mini Kit (Qiagen, Germantown, Md., U.S.A.), and 2 µl of solution containing poliovirus type II (Sabin strain, corresponding to approximately

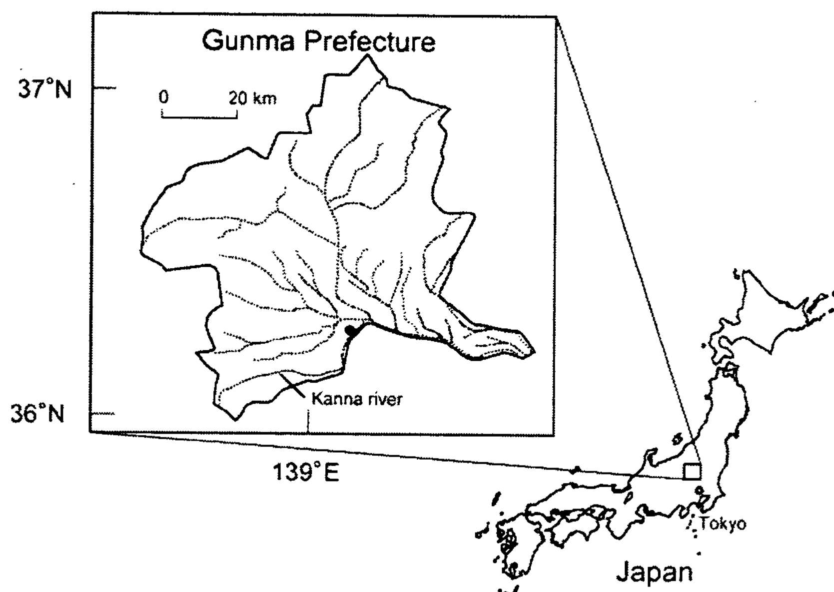

Fig. 1. Location of the sampling site in Gunma Prefecture, Japan. The dotted lines indicate the main river streams. The closed circle indicates the sampling site.

10^4 viral genes) was used as a control RNA extraction. The RNA was suspended in 60 µl of DNase/RNase-free water and then treated with 2 units of DNase I (TaKaRa, Tokyo). After reverse transcription, we used genogroup-specific primers (24) to amplify the partial capsid region of NoVs by reverse transcription-polymerase chain reaction (RT-PCR). The RT reaction was performed in 15 µl of DNase I-treated RNA solution and 15 µl of RT solution containing 1 mM of dNTP mixture, 10 mM of dithiothreitol, 0.05 g/liter of random hexamer (TaKaRa), 33 units of RNase inhibitor (TaKaRa), 300 units of reverse transcriptase (Superscript II, RNaseH (−); Invitrogen, San Diego, Calif., U.S.A.), and 4.5 µl of Superscript II buffer. The RT mixture was incubated at 42 C for 75 min and then at 99 C for 5 min. The following primers were used for the first PCR: 5′-CGY TGG ATG CGN TTY CAT GA-3′ (COG1F, sense), 5′-CCA ACC CAR CCA TTR TAC A-3′ (G1-SKR, antisense), 5′-CAR GAR BCN ATG TTY AGR TGG ATG AG-3′ (COG2F, sense), and 5′-CCR CCN GCA TRH CCR TTR TAC AT-3′ (G2-SKR, antisense). The following primers were used for the nested PCR: 5′-CTG CCC GAA TTY GTA AAT GA-3′ (G1-SKF, sense), 5′-CCA ACC CAR CCA TTR TAC A-3′ (G1-SKR, antisense), 5′-CNT GGG AGG GCG ATC GCA A-3′ (G2-SKF, sense), and 5′-CCR CCN GCA TRH CCR TTR TAC AT-3′ (G2-SKR, antisense) (16). We amplified the poliovirus type II VP1 gene using the following specific primers: 5′-AGC AAG CAC CGT ATT GAG CC-3′ (sense) and 5′-GTT TCA TGT CTG CTC CGT CTG-3′ (antisense) (24). The PCR protocol included incubation for 3 min at 94 C. This was followed by 40 cycles under each of the following sets of conditions: 94 C for 60 sec, at 50 C for 60 sec, and at 72 C for 2 min. Elongation was performed for 15 min at 72 C after the last cycle. This PCR procedure was repeated for the nested PCR using the inner primers (16). The size of the amplified DNA fragment was confirmed by 1.5% agarose gel electrophoresis. The DNA fragments was purified with a QIAquick PCR Purification Kit (Qiagen), and the nucleotide sequence was determined with an automated DNA sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Foster City, Calif., U.S.A.) using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

Real-time PCR. We quantified the NoV capsid genes using real-time PCR, as described previously by Kageyama et al. (11). The real-time PCR mixture contained 5 µl of cDNA (RT product), 2 µl of LightCycler Master Mix (Roche, Penzberg, Germany), 0.7 µM of each primer, and fluorogenic probes (probes for GI, 2 pmol of RING1(a)-TP and 2 pmol of RING1(b)-TP; probe for GII, 4 pmol of RING2-TP). The fluorogenic probes for real-time PCR were as follows: 5′-AGA TYG CGA TCY CCT GTC CA-3′ (RING1-TP(a)), 5′-AGA TCG CGG TCT CCT GTC CA-3′ (RING1-TP(b)), and 5′-TGG GAG GGC GAT CGC AAT CT-3′ (RING2-TP) (11). PCR amplification was performed with a LightCycler (Roche). The following PCR protocol was employed: 10 min at 95 C followed by 50 cycles under each of the following sets of conditions: 95 C for 10 sec, at 60 C for 25 sec, and at 40 C for 30 sec. Data were corrected using the NoV GI or GII cDNA plasmid standards described by Kageyama et al. (11).

Phylogenetic analysis. The capsid sequences of the reference strains of the NoVs were obtained from GenBank. The strains and accession numbers of these sequences are provided in the legend of Fig. 2. Phylogenetic analysis was performed as previously described by Katayama et al. (14). In brief, all of the NoV capsid region sequences (291 to 302 nt) were aligned using Clustal W (http://www.ddbj.nig.ac.jp/search/clustalw-j.html). A phylogenetic tree was constructed using the neighbor-joining (N-J) technique (26), and Kimura's two-parameter method (15) using the TreeExplorer (ver. 2.12) software (http://evolgen.biol.metro-u.ac.jp/). The reliability of the tree was estimated using 1,000 bootstrap replications.

Results

Detection and Quantification of NoV in C. fluminea

The NoV capsid genes detected by RT-PCR are shown in Table 1. NoV genomes were detected in 35 of the 58 samples (60%). Of these 35 samples, 6 (17%) samples contained GI amplicons alone and 15 (43%) samples contained GII amplicons alone. The other 14 (40%) samples contained both GI and GII amplicons. The detection rate was 40% to 100% every month except September, when all of the collected samples were negative. Predominance of any specific NoV genogroups detected in each tested month was not observed. We also quantified the number of copies of the NoV capsid gene using real-time PCR. In the 2 samples collected in July and December, 2.9×10^4 and 3.0×10^4 copies, respectively, of the GII genome were detected, while no GI genome was detected.

Phylogenetic Analysis of NoVs Detected in C. fluminea

We constructed the phylogenetic tree on the basis of the nucleotide sequence of the capsid genes using the N-J method (Fig. 2). The genogroup and genotype of norovirus genomes in corbiculas are shown in Table 1. The phylogenetic tree classified 20 and 29 strains into GI and GII clusters, respectively. In the present study,

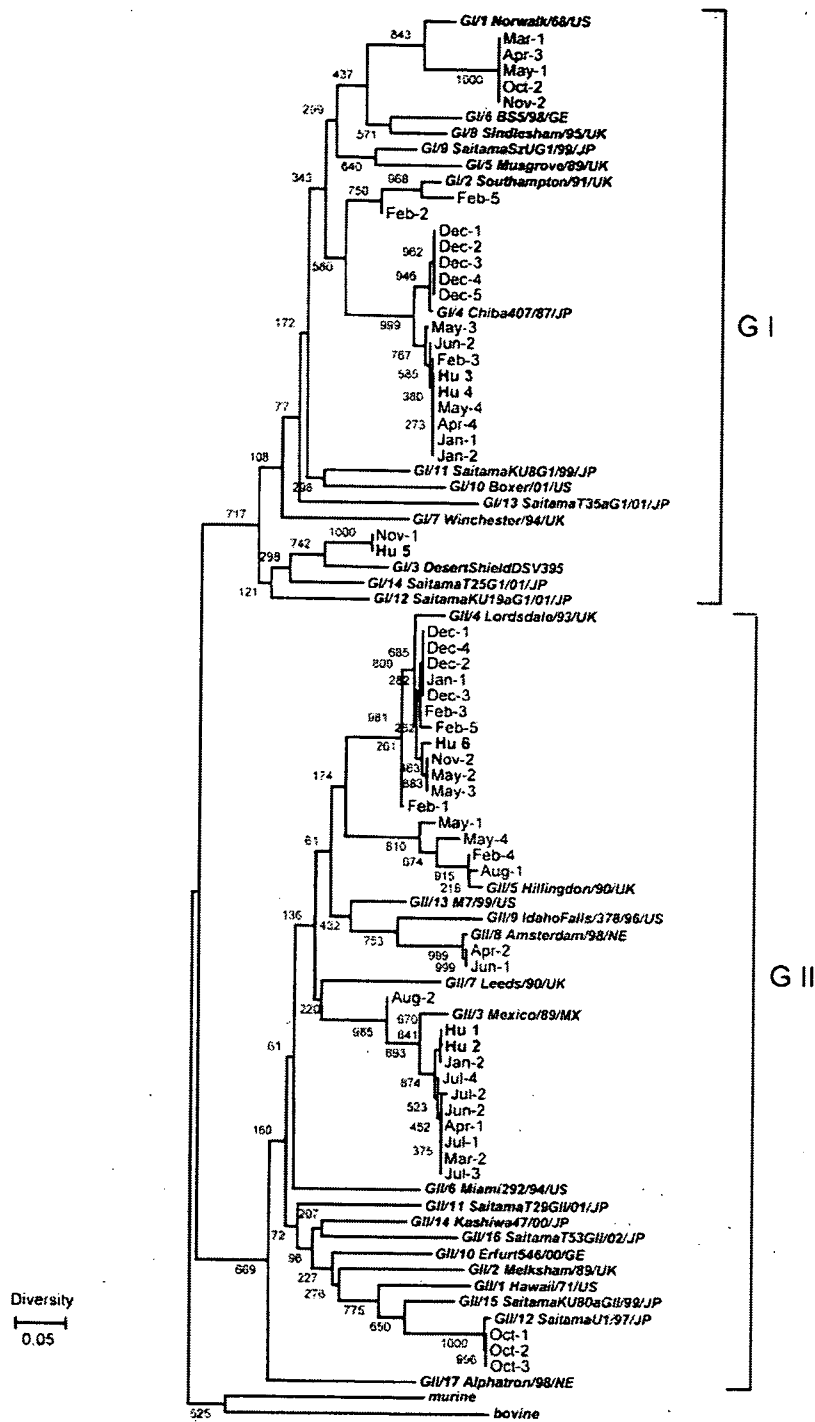

Fig. 2. Phylogenetic tree constructed based on the partial sequences of the norovirus (NoV) capsid gene. The distance was calculated using Kimura's two-parameter method, and the tree was plotted using the neighbor-joining (N-J) method. The numbers at each branch indicate the bootstrap value for the clusters supported by that branch. The accession numbers of our strains are also indicated in Table 1. "Hu1-6" refers to NoVs isolated from children with gastroenteritis who lived near the sampling site and are designated in boldface type. GI: genogroup I; GII: genogroup II. The following are the strain and GenBank accession numbers: Norwalk/68/US, M87661; BS5/98/GE, AF093797; Sindlesham/95/UK, AJ277615; SaitamaSzUG1/99/JP, AB039774; Musgrove/89/UK, AJ277614; Chiba407/87/JP, AB042808; Southampton/91/UK, L07418; Winchester/94/UK, AJ277609; SaitamaT35aGI/01/JP, AB112132; Boxer/01/U, AF538679; SaitamaKU8GI/99/J, AB058547; SaitamaK19aGI/01/J, AB058525; DesertShieldDSV395, U04469; SaitamaT25GI/01/JP, AB112100; Lordsdale/93/UK, X86557; Miami/292/94/US, AF414410; Alpha-

the GI strains were subdivided into 4 clusters, and the GII strains were subdivided into 5 clusters. The maximum genetic diversity (evolutionary distances calculated on the basis of substitutions per site of pairwise strains) within the GI and GII clusters was 0.36 and 0.38, respectively, while that between the GI and GII strains represented as an operational taxonomic unit (OTU) (26) was 0.47; the intercluster OTUs including the GI and GII strains from *C. fluminea* ranged from 0.005 to 0.112. Among the GI strains, 5 and 12 strains shared a

Table 1. Genogroup and genotype of norovirus genomes in corbiculas

Month	Samples	Genogroup(s)	Genotype(s)	GenBank accession nos.	
				GI	GII
January	Jan-1	GI + GII	GI/4, GII/4	AB246115	AB246135
	Jan-2	GI + GII	GI/4, GII/3	AB246116	AB246136
February	Feb-1	GII	GII/4	—	AB246137
	Feb-2	GI	GI/2	AB246117	—
	Feb-3	GI + GII	GI/4, GII/4	AB246118	AB246138
	Feb-4	GII	GII/5	—	AB246139
	Feb-5	GI + GII	GI/2, GII/4	AB246119	AB246140
March	Mar-1	GI	GI/1	AB246120	—
	Mar-2	GII	GII/3	—	AB246141
April	Apr-1	GII	GII/3	—	AB246142
	Apr-2	GII	GII/8	—	AB246143
	Apr-3	GI	GI/1	AB246121	—
	Apr-4	GI	GI/4	AB246122	—
May	May-1	GI + GII	GI/1, GII/5	AB246123	AB246144
	May-2	GII	GII/4	—	AB246145
	May-3	GI + GII	GI/4, GII/4	AB246124	AB246146
	May-4	GI + GII	GI/4, GII/5	AB246125	AB246147
June	Jun-1	GII	GII/8	—	AB246148
	Jun-2	GI + GII	GI/4, GII/3	AB246126	AB246149
July	Jul-1	GII	GII/3	—	AB246150
	Jul-2	GII	GII/3	—	AB246151
	Jul-3	GII	GII/3	—	AB246152
	Jul-4 [a]	GII	GII/3	—	AB246153
August	Aug-1	GII	GII/5	—	AB246154
	Aug-2	GII	GII/3	—	AB246155
October	Oct-1	GII	GII/12	—	AB246156
	Oct-2	GI + GII	GI/1, GII/12	AB246127	AB246157
	Oct-3	GII	GII/12	—	AB246158
November	Nov-1	GI	GI/3	AB246128	—
	Nov-2	GI + GII	GI/1, GII/4	AB246129	AB246159
December	Dec-1	GI + GII	GI/4, GII/4	AB246130	AB246160
	Dec-2 [a]	GI + GII	GI/4, GII/4	AB246131	AB246161
	Dec-3	GI + GII	GI/4, GII/4	AB246132	AB246162
	Dec-4	GI + GII	GI/4, GII/4	AB246133	AB246163
	Dec-5	GI	GI/4	AB246134	—
Total	35	49			

Samples collected in September were negative.
[a] The genome in the 2 samples were quantified: Jul-4, 2.9×10^4 copies (GII) and Dec-2, 3.0×10^4 copies (GII). Units are copy numbers of NoV genome /g of the gills and digestive diverticula.

tron/98/NE, AF195847; Mexico/89/MX, U22498; Leeds/90/UK, AJ277608; Amsterdam/98/NE, AF195848; Idaho-Falls/378/96/US, AY054299; M7/99/US, AY130761; Kashiwa47/00/JP, AB078334; SaitamaT53GII/02/JP, AB112260; SaitamaT29GII/01/JP, AB112221; Hawaii/71/US, U07611; SaitamazU1/97/JP, AB039775; SaitamaKU80aGII/99/JP, AB058582; Melksham/89/UK, X81879; Erfurt/546/00/GE, AF427118; Hillingdon/90/UK, AJ277607; HU1, AB246164; HU2, AB246165; HU3, AB246166; HU4, AB246167; HU5; AB246168; HU6, AB246169; Murine norovirus 1, AY228235; Bovine enteric calicivirus, AJ011099.

close genetic relationship with GI/1 and GI/4, respectively. In addition, two GI/2 and one GI/3 strains were found. The GII strains from *C. fluminea* were classified into the following 5 clusters: GII/3–5, GII/8 and GII/12. Most (20 strains) of the GII strains belonged to either GII/3 or GII/4.

During the investigation period, we observed that NoVs from children with gastroenteritis who lived near the sampling site (within approximately 5 km) were genetically related to the strains isolated from *C. fluminea* (Fig. 2). Thus, these results suggest that the NoVs isolated from *C. fluminea* showed a wide diversity in their capsid genes, and that human strains were genetically related to the strains detected in *C. fluminea*.

Discussion

NoVs have been detected in seawater bivalves such as oysters and clams in France (7), the United States (2, 8), the United Kingdom (4, 17), Spain, Sweden (5), and Japan (24). These viruses have been associated with human gastroenteritis. NoV was detected in approximately 5%–20% bivalves, including oysters and clams. We found that approximately 60% of the corbicula clams collected from the river in Gunma Prefecture in 2004 had the NoV genome, and more than 10^4 copies of the NoV genome were detected in approximately 5% of the clams. We previously reported that NoVs were detected in approximately 10% of Japanese oysters, and that 5% of the oysters showed relatively high concentrations of the NoV genome (24). Thus, it is suggested that the prevalence of NoV in the corbicula clams in rivers in Japan is higher than that in seawater bivalves.

It is well known that NoV detected in infected patients and bivalves can be classified into 2 major genogroups (GI and GII), and that each genogroup can be further subdivided into many clusters. The genetic diversity ranges from 0.12 to 0.36 among GI strains, from 0.12 to 0.46 among GII strains and from 0.46 to 0.49 between GI-GII strains (12, 14). The phylogenetic tree showed that the NoV in clams can be classified into 4 GI clusters and 5 GII clusters. Genetic diversity among the GI strains, among the GII strains, and between the GI-GII strains was 0.36, 0.38 and 0.47, respectively (Fig. 2). Furthermore, the rate of nucleotide substitutions in each intercluster of GI and GII was in the range of 0.50 and 11.2%. This suggests a wide genetic diversity of NoV in the corbicula clams in rivers, similar to that in oysters and clams (1, 16, 30).

The NoV capsid genes from 6 stools of gastroenteritis patients who lived near the sampling site were genetically related to the strains detected in corbicula clams, even though the NoV capsid genes were only partially sequenced. Previous studies have shown that the NoV genogroups detected in oysters and humans are slightly different. For example, although both GI and GII have been detected in oysters, only GII has been detected in humans (6, 24). A recent study demonstrated the existence of GII/4 in oysters during several outbreaks of NoV infection in various countries, including the Netherlands, the United States and Japan (7, 25, 27). In this study, we detected many NoV genotypes from the corbicula clams in the river. They were similar to those detected in other seawater bivalves. Especially, it is remarkable that 4 genotypes including GII/4 were also detected in patients who lived near the sampling site. Thus, it is suggested that the corbicula clams collected from the river in Gunma Prefecture were highly contaminated with genetically diverse NoV strains. The possibility of the existence of 2 or more NoVs of the same genogroup in a single specimen cannot be completely excluded. A cloning might be required for a detailed analysis of the NoV genotype in bivalves.

Several viruses such as NoVs or hepatitis virus type A might be present in the stools of gastroenteritis or hepatitis patients. Seawater, sewage and river water may have been polluted with these viruses (18, 21, 29). Because various types of bivalves are capable of indiscriminately accumulating viruses and may concentrate them (2, 5, 7), it is possible that these bivalves cause the main infection. Asian clams including *C. fluminea* are marketed as fresh food imports into Japan for human consumption. In Japan, various corbicula clams are almost always consumed after heating and boiling. However, the boiling required to open the bivalves is not necessarily sufficient to inactivate viruses (23).

The present study suggests that *C. fluminea* in the river might be a source of NoV infection, as are other seawater bivalves such as oysters and clams, and that this finding identifies an important etiological factor of gastroenteritis.

The prevalence of NoV in various bivalves has not yet been elucidated. To the best of our knowledge, there are no reports regarding the detection, quantification or phylogenetic analysis of NoVs in clams. Thus, molecular epidemiologic studies on NoVs in bivalves, including corbicula clams in rivers, and in humans are necessary to provide important data for a more detailed assessment of NoV infections and their regional prevalence, and the risk factors of NoV, as well as to provide an estimate of NoV pollution in rivers.

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Short communication

Statistical analysis of attack rate in norovirus foodborne outbreaks

Mamoru Noda [a,*], Shinji Fukuda [b], Osamu Nishio [c]

[a] *Division of Biomedical Food Research, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo, 158-5801, Japan*
[b] *Center for Public Health and Environment, Hiroshima Prefectural Institute of Science and Technology, 1-6-29, Minami-machi, Minami-ku, Hiroshima City, 734-0007, Japan*
[c] *National Institute of Infectious Diseases, 4-7-1, Gakuen, Musashimurayama City, Tokyo 208-0011, Japan*

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Abstract

Norovirus (NoV), which causes foodborne gastroenteritis outbreaks, is one of the important viruses in public health. We statistically analyzed the attack rate in foodborne outbreaks caused by NoV. The attack rate in 95 oyster-associated outbreaks was significantly higher than that in 195 food handler-associated outbreaks ($P=0.007$). The difference in the number of NoV genotypes implicated is considered to be an important factor for this difference. The attack rate in 20 outbreaks associated only with GII/3 was higher than that in 143 other outbreaks ($P=0.247$), while the attack rate in 27 outbreaks associated only with GII/4 was lower than that in 136 other outbreaks ($P=0.004$), suggesting that GII/4 NoVs cause asymptomatic infection more frequently than do other NoV genotypes. Our results suggest that differences in implicated foods, susceptibility of the host to NoV infection, and pathogenicity of NoVs may influence the attack rate in NoV foodborne outbreaks.
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Keywords: Attack rate; Foodborne outbreaks; Food handler; GII/4; Norovirus; Oyster; Statistical analysis

1. Introduction

In recent years, viruses have been increasingly recognized as important causes of outbreaks of foodborne disease (Fleet et al., 2000; Graczyk and Schwab, 2000; Koopmans and Duizer, 2004; Parashar and Monroe, 2001). Norovirus (NoV) is currently recognized as the most important foodborne virus, which causes gastroenteritis outbreaks. The foods affected can be classified into two distinct groups based on the route of contamination: one group includes bivalve shellfishes such as oysters, which are contaminated with NoV in their sea life (Boxman et al., 2006; Cheng et al., 2005; Nishida et al., 2003; Nishida et al., 2007; Saito et al., 2006; Ueki et al., 2004, 2005), and the other group includes various kinds of foods other than bivalve shellfishes, which are secondarily contaminated with NoV from infected food handlers during food processing and/or food serving. Despite the fact that oysters are the most important issue for the prevention and control of NoV in foods, there is no virological standard for oysters intended for raw consumption in Japan and other countries (European Commission Health & Consumer Protection Directorate-General, 2002; Nishida et al., 2003; Nishida et al., 2007). Although risk analysis based on scientific data must be performed before setting the virological standard, there is a lack of scientific data on attack rate, which are required to calculate the minimum virus amount needed for infection when oysters are eaten, as well as a lack of data on the numbers of infectious NoV particles in oysters involved in foodborne outbreaks. Although the infectious dose of NoV is estimated to be about 10 particles at least (URL: http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus-factsheet.htm), the number is not necessarily equal to that in outbreaks associated with oyster consumption and might depend on each individual or each virus strain. The attack rate may be influenced by factors other than amount of infectious virus particles ingested, such as host susceptibility and virus pathogenicity. In an initial investigation to obtain data that can be used for risk analysis for the prevention and control of NoV in food, we statistically compared the attack rates in oyster-associated outbreaks and food handler-associated outbreaks and the attack rates in outbreaks caused by different NoV genotypes.

* Corresponding author. Tel.: +81 3 3700 1141.
E-mail address: mamorunoda@nihs.go.jp (M. Noda).

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2. Materials and methods

2.1. Subjects

A total of 290 foodborne outbreaks that occurred between April 2001 and January 2005 in various areas of Japan were subject to statistical analysis. In all outbreaks, NoVs were detected by reverse-transcription, (nested) PCR and/or real-time PCR commonly performed in Japan (Kageyama et al., 2003; Kojima et al., 2002; Nishida et al., 2003; Nishida et al., 2007; Ueki et al., 2004; Ueki et al., 2005) with or without some modifications depending on laboratories, and NoVs were concluded to be the causal agent responsible for each outbreak. Among the 290 outbreaks, NoVs were genotyped in 163 outbreaks after partial sequencing of the capsid region (Nishida et al., 2003; Nishida et al., 2007). In most cases, sequencing was performed directly without cloning of the PCR products. Some PCR products, most of which were amplified from oyster samples, were cloned before sequencing because oysters may include various NoV strains. Phylogenetic analysis was performed for genotyping using the reference strains reported by Katayama et al. (Katayama, 2004).

2.2. Statistical analysis

We statistically compared the attack rate (ratio of the number of patients with symptoms such as nausea, vomiting, diarrhea, abdominal pain, and/or fever to the number of the individuals that have eaten foods suspected as being responsible for each outbreak) and the number of the involved patients in 95 oyster-associated outbreaks and 195 food handler-associated outbreaks by using the Mann–Whitney U test. The oyster-associated outbreaks included not only outbreaks in which NoV was detected from implicated oysters but also outbreaks in which oysters were included in the menu suspected as the food vehicle without the detection of NoV from the oysters. Food handler-associated outbreaks included outbreaks in which NoV was detected from food handlers in the facilities that were suspected as being responsible for the foodborne outbreaks. In some food handler-associated outbreaks, the food vehicle was not determined.

In 163 outbreaks in which NoVs were detected and genotyped, GII/4 and GII/3 were the genotypes most frequently associated with foodborne outbreaks (Table 1). We therefore compared the attack rates in outbreaks in which only GII/4 was detected (27 cases) and other outbreaks (136 cases) and the attack rates in outbreaks in which only GII/3 was detected (20 cases) and other outbreaks (143 cases) by using the Mann–Whitney U test.

3. Results

3.1. Comparison of oyster-associated outbreaks and food handler-associated outbreaks

The median attack rates were 58.3% in the 95 oyster-associated outbreaks and 47.2% in the 195 food handler-associated outbreaks (Table 2), the difference being statistically significant ($P=0.007$). This result indicates that the attack rate in oyster-associated outbreaks is higher than that in food handler-associated outbreaks. The median numbers of patients were 17 in the oyster-associated outbreaks and 40 in the food handler-associated outbreaks, indicating that the scale of food handler-associated outbreaks tends to be larger than that of oyster-associated outbreaks, though there was no statistical difference between them (Table 3).

Table 1
Number of foodborne outbreaks by NoV genotypes

Genotype	Cases detected single genotype		Cases detected two or more genotypes		Total
	Oyster-associated outbreaks	Food handler-associated outbreaks	Oyster-associated outbreaks	Food handler-associated outbreaks	
GII/3	4	16	20	6	46
GII/4	4	23	12	5	44
GII/5	2	3	17	5	27
GI/4	3	4	12	3	22
GII/12	1	2	9	3	15
GII/6	1	6	5	2	14
GI/8	0	1	7	3	11
GI/7	1	1	8	0	10
GII/15	0	2	8	0	10
GI/1	0	1	5	3	9
GII/14	0	0	8	1	9
GI/2	1	2	4	1	8
GII/2	1	6	0	1	8
GII/1	0	2	2	3	7
GII/8	0	2	3	1	6
GI/13	0	0	5	0	5
GI/11	0	0	2	2	4
GI/12	0	0	3	1	4
GI/14	0	0	4	0	4
GI/5	1	0	3	0	4
GI/9	0	0	3	0	3
GII/10	0	1	0	1	2
GII/11	0	0	2	0	2
Others	1	0	4	0	5

3.2. Comparison between different genome types of NoVs

We compared the attack rates in outbreaks caused by different NoV genotypes. The median attack rates were 41% in the 27 outbreaks in which only GII/4 was detected and 56.9% in the other 136 outbreaks (Table 4), the difference being statistically significant ($P=0.004$). This result indicates that the attack rate in GII/4 cases is lower than that in other NoV genotype cases. On the other hand, the median attack rates were 64.8% in the 20 outbreaks in which only GII/3 was detected and 53.2% in the other outbreaks ($P=0.247$), indicating that the attack rate in GII/3 cases is higher than that in other NoV genotype cases (Table 5).

4. Discussion

In this study, we showed that the attack rate in oyster-associated outbreaks was significantly higher than that in food

Table 2
Comparison of attack rate between oyster-associated outbreaks and food handler-associated outbreaks

Group	Number of cases	Attack rate (%)		
		Median	25th percentile	75th percentile
Oyster-associated outbreaks	95	58.3*	40	75
Food handler-associated outbreaks	195	47.2*	33.3	67.7

*: The difference between the median attack rates were statistically significant ($P=0.007$) by using the Mann–Whitney U test.

Table 4
Comparison of attack rate between GII/4-associated outbreaks and other outbreaks

Group	Number of cases	Attack rate (%)		
		Median	25th percentile	75th percentile
GII/4-associated outbreaks	27	41*	29.9	54.5
Other outbreaks	136	56.9*	40	75

*: The difference between the median attack rates were statistically significant ($P=0.004$) by using the Mann–Whitney U test.

handler-associated outbreaks. The reason may be explained as follows from the viewpoint of the difference in contamination route. First, in the oyster-associated outbreaks, one or more NoV strains to which each person is susceptible might have selectively grown in its intestinal cells after the oyster-consumption because oysters might accumulate various NoV strains during their sea life. In contrast, most of the food handler-associated outbreaks were caused by a single NoV strain that had contaminated the foods after propagation in the food handlers. Some consumers, therefore, are not susceptible to an NoV strain because of the lack of the receptor(s) for it (Huang et al., 2003; Huang et al., 2005; Larsson et al., 2006; Marionneau et al., 2002; Tan and Jiang, 2005). Indeed, a single NoV genotype was responsible for most of the food handler-associated outbreaks, whereas multiple NoV genotype strains were frequently involved in the oyster-associated outbreaks (Table 1). Second, the amount of NoV particles in foods that might have been contaminated from food handlers during the food processing and/or food serving might vary, and some of the implicated food might not have been contaminated with NoV. The number of patients in the food handler-associated outbreaks tended to be more than that in the oyster-associated outbreaks. This may be explained by the difference in involved facilities; most of the facilities associated with oyster-associated outbreaks were small restaurants, while most of the facilities associated with food handler-associated outbreaks were large restaurants such as hotels serving food for parties and schools.

The attack rate in the oyster-associated outbreaks varied between 40% (25th percentile) and 75% (75th percentile), and the median attack rate was 58.3% (Table 2). Therefore, some individuals did not develop any symptoms despite the fact that they had consumed oysters suspected of being the food vehicle. In the oyster-associated outbreaks, susceptibility of the host to NoV infection might have been one of the reasons but was not thought to be a major factor because some NoV strains to which the host is susceptible included in oysters could selectively propagate in individuals as stated above. There are three other possible reasons. First, some individuals might have asymptomatic infection. Second, some individuals might have blocked the NoV infection by their immunity. Third, the oysters eaten by individuals who were not affected might have been less or not contaminated with NoVs because it has been reported that oysters packed in the same package for sale or harvested from the same balsa raft in the cultivating sea area have different copy numbers of the NoV genome and that some oysters selected from the same lot in which NoV was detected in other oysters do not include NoV (Nishio et al., 2004; Noda et al., 2004).

Recent studies have suggested that NoVs use a histo-blood group antigen(s) expressed on intestinal epithelial cells as their receptor and that different NoV strains may use different types of histo-blood group antigen (Huang et al., 2005; Tan and Jiang, 2005). These reports suggest that the attack rate may depend on difference of genotype. GII/4 is thought to be able to infect all secretor individuals, suggesting that susceptibility to GII/4 appears to be greater than that to other NoV genotypes. From this viewpoint, we compared the attack rate in outbreaks associated with different genotypes. Unexpectedly, the attack rate in outbreaks associated with GII/4 genotype was lower than that in outbreaks associated with other NoV genotypes, while the attack rate in outbreaks associated with GII/3 genotype was higher than that in outbreaks associated with other NoV genotypes. The low attack rate in the GII/4 cases can not be explained only by the fact that GII/4 was more frequently associated with the food handler-associated outbreaks than were other NoV genotypes (Table 1) because GII/3 has the same tendency as GII/4 and the attack rate in the GII/4 cases was lower than that in the food handler cases (Tables 2, 4). Therefore, it is possible that GII/4 causes asymptomatic infection more frequently than do other NoV genotypes. This

Table 3
Comparison of number of patients between oyster-associated outbreaks and food handler-associated outbreaks

Group	Number of cases	Number of patients		
		Median	25th percentile	75th percentile
Oyster-associated outbreaks	95	17	7	39
Food handler-associated outbreaks	195	40	12	108

Table 5
Comparison of attack rate between GII/3-associated outbreaks and other outbreaks

Group	Number of cases	Attack rate (%)		
		Median	25th percentile	75th percentile
GII/3-associated outbreaks	20	64.8*	40.2	81.7
Other outbreaks	143	53.2*	37.6	73.8

*: The difference between the median attack rates were statistically significant ($P=0.247$) by using the Mann–Whitney U test.

hypothesis is supported by the fact that GII/4 was frequently detected from asymptomatic in-hospital patients and staff in a study by Gallimore et al. (2004). This unique characteristic of GII/4 may be responsible for the recent increase in outbreaks caused by GII/4 strains, especially in hospitals, elderly home facilities, residential facilities, and nursing homes, all over the world (Bull et al., 2006; Ike et al., 2006; Lopman et al., 2004; Okada et al., 2006; Siebenga et al., 2007; Wu et al., 2006; Yoshizumi et al., 2005). The high attack rate in the GII/3 cases was also unexpected because binding ability of GII/3 strains to secretor individuals with blood type O and non-secretor individuals was shown to be weak in a saliva binding assay (Huang et al., 2005; Tan and Jiang, 2005). In recent years, GII/3 genotype as well as GII/4 has been frequently associated with gastroenteritis outbreaks (Bull et al., 2006; Gallimore et al., 2004; Wu et al., 2006). GII/3 strains may have high pathogenicity, transmissibility, or infectivity. We may not exclude the influence of mass immunity for difference in attack rate between different genotypes. However, secretory IgA antibodies specific for NoVs, which can block NoV infection, exist in intestinal tracts for a short period after the recovery from illness (Nishio et al., 1988). Mass immunity, therefore, does not considerably affect our results of analysis in attack rate between different genotypes.

In conclusion, we showed in this study that there were differences between attack rates in oyster-associated outbreaks and food handler-associated outbreaks and between attack rates in GII/4- or GII/3-associated outbreaks and other NoV genotype-associated outbreaks. These results show that the attack rate in NoV foodborne outbreaks may be influenced by differences in implicated foods, susceptibility of the host for NoV infection, and pathogenicity of NoVs. To estimate the minimum amount of virus particles in oysters required for infection, it is necessary to investigate foodborne outbreaks epidemiologically in detail and to perform genotyping of NoVs involved for obtaining data on attack rate as well as quantitation of copy numbers of NoV genomes, or titration of infectious NoV particles if possible (Straub et al., 2007), in the foods such as oysters, involved in foodborne outbreaks. Our results should be useful for risk analysis for the prevention and control of NoV in food.

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ノロウイルス感染症 ①

ノロウイルス感染症

西尾 治

ノロウイルスは1997年に食中毒病因物質に加えられたことから，食中毒を起こすウイルスとの認識が強く，感染症は軽視されていた．ところが，2004年末から2005年の初めに，ノロウイルスによる感染性胃腸炎の集団発生が高齢者特別養護施設で全国的に多発し，社会的にも大問題となった．当時，ノロウイルスが聞き慣れなく，新たに出現したウイルス，殺人ウイルス如きの誤った情報が一部で言われた．ノロウイルスは約40年前に発見され，名前が新しいだけで，死亡例は極めて稀である．

2年後の2006年11月から2007年の初めに，小児科，学校，高齢者施設等でノロウイルスによる感染性胃腸炎のヒト-ヒト集団発生，および食中毒事件が全国的に多発し，共に過去最大となった[1]（**図1**）．ノロウイルスによる感染症と食中毒は表裏一体であり，両面からの対策が必要である．

ノロウイルスの歴史

ノロウイルスは1968年にアメリカ，ノーウォークで急性胃腸炎患者の糞便から電子顕微鏡で発見された．形態的特徴は小さく，球形を呈し，表面に構造が見られることから小型球形ウイルス(SRSV)と呼称されていた．2002年8月国際ウイルス命名委員会でカリシウイルス科，ノロウイルス属と命名された．

ノロウイルスの概要

ノロウイルスは直径30～40 nm前後で球形を呈し，表面はカップ状の蛋白構造物で覆われ，その内部に長さ約7.7 kbのプラス1本鎖RNA分子ゲノムを持つ．エンベロープはない．遺伝子型が多く，genogroup(G) I とⅡに分けられ，34以上の型が存在している．増殖系(組織培養，実験動物)は未だ見出されていないことが，ノロウイルスの感染性，血清型，消毒効果は正確な意味で明らかでない．最近，腸管細胞の3D培養により感染性の検査法が開発されたものの[2]，日常的に用いるには難しく，簡便な増殖法の確立が望まれる．

ノロウイルスの特性

感染力が非常に強く，ウイルス粒子10～100個で感染・発病させることができる．

ノロウイルスに類似のネコカリシウイルスから推測すると，物理化学的抵抗性は強い．ノロウイルスは乾燥・液中で長期間安定であり，4℃では2か月間，20℃では1か月間程度生存可能と考えられる[3]（**図2**）．pH3～10では短時間で不活化されない．

ノロウイルスの不活化には85℃ 1分間あるいは70℃で5分間の加熱が必要である．70％アルコールでの不活化には5分間以上要し，噴霧あるいは単に70％アルコールで拭く程度ではノロウ

にしお おさむ：国立感染症研究所感染症情報センター
連絡先：〒208-0011 東京都武蔵村山市学園4-7-1 国立感染症研究所村山庁舎

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イルスを完全に殺滅できない．

塩素濃度3～6 ppmでは短時間で不活化されない．

免疫

ノロウイルスの感染防御には腸管のIgA抗体が重要な役割を担う．このIgA抗体は持続が短く，数か月で消滅し，同じ遺伝子型に再感染する．得られた抗体は他の遺伝子型には感染を防御しないので，他の遺伝子型に感染・発病する．したがって，乳幼児から高齢者に至るまで，何度でも感染・発病を繰り返す．

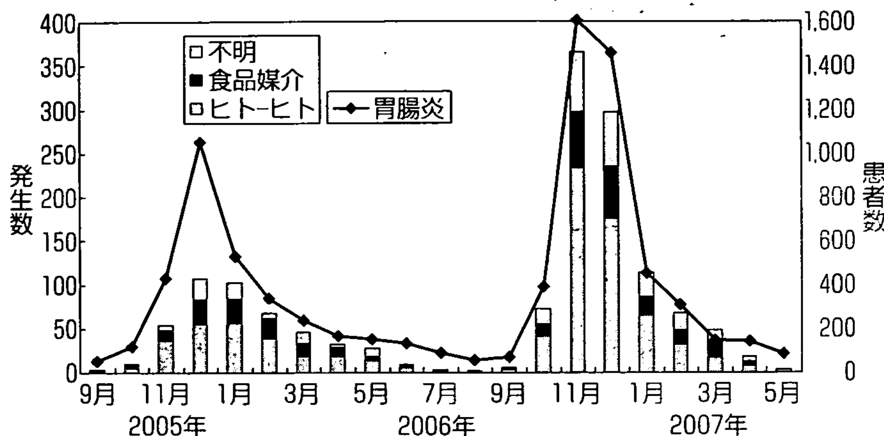

図1　月別のヒト-ヒト感染集団発生，食中毒事件，原因不明集団発生数ならびに感染性胃腸炎患者からのノロウイルス検出数(2005年9月～2007年5月)(病原微生物検出情報，2007年5月31日)

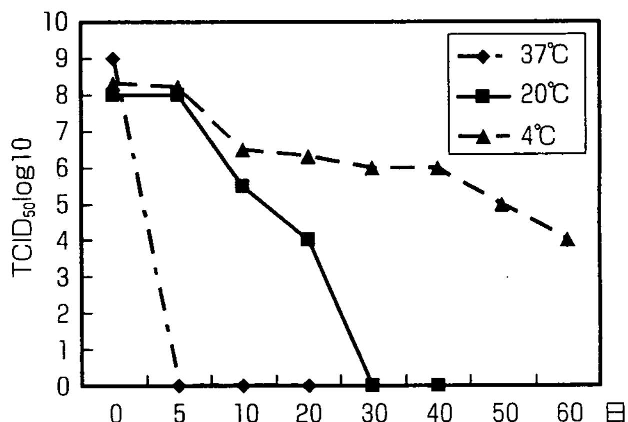

図2　ネコカリシウイルスの乾燥状態での温度による感染性の持続日数

(Doultree JC : J Hos Infect **41** : 51, 1999を改変)

ノロウイルスによる感染性胃腸炎の発生状況

感染症法では感染性胃腸炎は最も軽い5類感染症である．厚生労働省の感染症発生動向調査で，「感染性胃腸炎」は全国の定点(3,000か所の小児科病院または診療所)から，毎年90万程度の患者数が報告されている．感染性胃腸炎はノロウイルスの他に，サポウイルス，アストロウイルス，アデノウイルス，エンテロウイルス，細菌，原虫等によっても起きる．感染性胃腸炎患者の病原体検査は完全に行われていないので，正確にはわからないが，感染性胃腸炎患者の20%程度はノロウイルスと推定され，10倍すると実数に近いとされ，例年では180万人程度で，昨年は2倍の約360万人が小児科領域でのノロウイルスによる感染性胃腸炎患者と推定されている．

ノロウイルスの感染様式

ノロウイルスはヒトの小腸でのみ感染し，そこで増殖する．感染者の糞便・嘔吐物からウイルスが排泄され，それが口に入り感染する．環境中，食品中で増殖することはない．

ノロウイルスによる感染症の集団発生，食中毒事件は突然起きるものではない．その前にノロウイルス感染者が必ず存在し，その感染者が身の周りを汚染すれば，感染症の集団発生が，食品を汚染すると食中毒となる．感染者が環境と食品を同時に汚染すると両方が同時に起こり，判断が困難となる．そのような事例も多く見られる．

1. ヒト-ヒト感染

ノロウイルス感染源は感染者の排便後にウイルスが手に付いたり，嘔吐物を処理する際に，消毒しないと雑巾，バケツ，洗い場等を汚染し，手にも付く．汚染された場所を触れることによりウイルスが手に付き，食事の際(サンドイッチ，おにぎり，果物等)に手を介して口に入り，感染が成立する．

表1に高齢者施設での集団発生後の拭取り検査

表1　集団発生施設内のウイルス汚染状況

場所	コピー数(/cm²)
トイレの便座	520〜15,000
手すり	110〜5,900
ドアノブ	120〜270

成績を示したが，いずれの場所も大量のノロウイルスに汚染されており，われわれの身の周りは糞便にまみれた状況にあると言える．

2. 空気感染

ノロウイルスに汚染された糞便，嘔吐物が乾燥すると舞い上がり，塵となり，糞便・吐物には大量のウイルスが存在することから，空気中にものすごい量のウイルスが漂う．いったん舞い上がるとノロウイルスは極めて微小のため，なかなか落下せず，長時間空中に漂う．舞い上がったウイルスが直接口に入る，あるいは食べ物，衣類に付き，そして手に付き，口に入り感染する．ノロウイルスの流行期は冬期であり，窓を閉めているため，舞い上がったウイルスはなかなか外に出て行かない．近年は空調が発達しており，空気が常に動いており，ウイルスは広範囲に撒布され，多くのヒトが感染することになる．さらにウイルスが空調のフィルターに付着すると，そこが感染源となり3週間程度は撒布され，患者発生が続くこともある．

学校等では体育館，講堂での行事の後に，ノロウイルスの集団発生が起きることがある．以前に，その場所が嘔吐物あるいは糞便を介して汚染されていたことが推測され，使用前に換気と床を消毒する．

3. 食品を介する感染

1) 食品取扱者・調理者による食中毒事件

ノロウイルスに感染した食品取扱者・調理者がノロウイルスに汚染された糞便・吐物を食品に付着させることにより，食中毒事件となる．食品を提供する直前にウイルスが付着した手で直接，刺身，寿司，サラダ，パン，和え物等を取り扱ったことにより起きている．近年はこの食中毒事件が多発しており，しばしば施設，学校等で患者数が100人以上で発生している．手に大量のウイルスが付着し，多数の患者を感染させている．

2) 汚染されたカキ等の二枚貝を介するもの

ノロウイルスによる感染性胃腸炎患者の糞便・吐物から膨大な量のウイルスが排出され，ノロウイルスに汚染された糞便等は便器に流され，ウイルスは下水処理場へ行き，ごく一部が河川水，海域を汚染する．ウイルスは海水と共に二枚貝(カキ，シジミ，アサリ，ハマグリ等)の中腸腺に濃縮・蓄積する．ウイルスに汚染された二枚貝を生あるいは加熱不足で食することにより，食中毒となる[4]．

3) 環境汚染における飲料水汚染による食中毒

近年，飲料水(井戸水，簡易水道)による事件が発生している[5]．飲料水が汚染されると，患者発生が連日見られる．事件を起こした井戸の深さは10m以内で，浅井戸は特に注意が必要である．水源では排便，嘔吐をさせない環境対策を行い，ヒトは環境を守る義務がある．

臨床症状

経口感染で，潜伏期間は通常12〜72時間，主症状は嘔気，嘔吐，下痢，腹痛である．感染部位は小腸の粘膜上皮細胞で，腸管の炎症に伴う下痢症状を呈し，激しい水様性の便が数回続くこともある．胃の病理学的病変は認められないが，内容物を腸に送る運動神経の機能低下・麻痺に伴う嘔気，嘔吐の症状が見られる．

ノロウイルスによる嘔吐は突然，急激に強く起こるため，トイレに行く時間がなく，所構わず嘔吐する．その際に腸内容物が逆流し，ウイルスが吐物中に入り込み，感染源となる．このことがノロウイルスの感染拡大防止を困難にしている．症状は1〜2日続いた後治癒し，後遺症は残さない．不顕性感染は30%程度と考えられている．

高齢者，乳幼児等の抵抗力の弱いヒトでは，脱水症状を起こすことがある．嘔吐により誤嚥性肺炎や窒息を起こし，重症化することがあるので，健康観察をしっかり行う．高齢者や体力の弱いヒトの嘔吐時には，嘔吐物が詰まっていないかを確

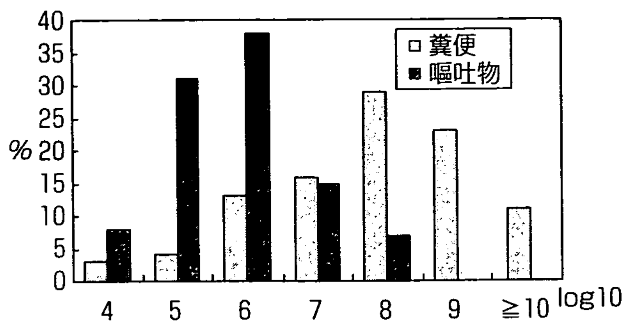

図3 急性期の患者の糞便および嘔吐物中のウイルス量(コピー数/g)

認する．詰まっているときには背中を手で数回叩き，嘔吐物を吐き出させる．出ないときには嘔吐物を指で掻き出し，直ちに医師や看護師を呼ぶ．不在の時には救急車を呼ぶか，病院に連れて行く．

ノロウイルスに直接効果のある薬剤はなく，脱水症状が強いときには補液などの対症療法を行う．下痢止めは症状を長引かせるので，極力避ける．ワクチンは存在していない．

多くの患者の糞便1g中には1億個以上，吐物では1g中に百万個以上のウイルスが存在する(**図3**)．

症状の消失後，10日間程度，長いときには1か月ほど，ウイルスの排出が続く．この間は手洗いの徹底，介護業務，食品を直接取り扱わない等の感染拡大防止を行う．

下痢等の症状がなくてもウイルスを糞便から排出することがあり(不顕性感染)，患者と同様にウイルスを大量に排出するヒトも見られる[4]．ヒトは常にノロウイルスを排出する可能性がある．家族，職場等で患者がいる時には，自身も感染する危険性が高いので，健康管理に特に注意する．

近年の流行株

2004年以降，世界的にノロウイルスが流行し，集団発生が多発している．主流株はGⅡ/4株であり，2002年以降ポリメラーゼ領域で，2年ごとに変異株が出現し(2002年型，2004年型，2006年a，b型)，変異が起きた年に大流行が発生している．遺伝子の変異領域からウイルスの増殖性が旺盛になっていると推測される．最近，ウシ，ブタからGⅡ/4株を検出し，ブタ/ヒトまたはウシ/ヒトのノロウイルス間で遺伝子組替えが起こり，親和性や病原性が変化した株の出現する可能性が示唆され[6]，動物由来の組換え株の監視が必要である．

表2 ノロウイルス検査法の検出感度

検査法	感度(/ml)*
電子顕微鏡	＞100万
RT-PCR	＞100～1000
リアルタイムPCR	＞100～1万
ELISA法	＞100万

*：1ml中に含まれるウイルス量，それぞれの検査法で陽性となる最小のウイルス量

診断法

ヒトの検査材料は糞便あるいは嘔吐物を検査する．二枚貝は中腸腺を，その他の食材はヒトが直接，ノロウイルスを付けているので，野菜，刺身等の表面が水に溶けないものは表面を洗い，洗った液を，洗えないものは表面を薄く削り取り検査する．

検査法はRT-PCRあるいはリアルタイムPCRで行うのが望ましい．ただし，RT-PCRでは増幅された遺伝子について，ハイブリダイゼーションで確認するか，あるいは遺伝子配列を決定して，ノロウイルスのクラスターに属することを確認する(詳細は厚生労働省のノロウイルス検出法 http://www.mhlw.go.jp/topics/syokuchu/kanren/kanshi/031105-1.html を参照)．

電子顕微鏡およびELISA法では1ml中にウイルス粒子が100万個以上存在しないと陽性ならない．両検査での陰性はウイルスが存在しないことを必ずしも表していなく，両検査で陰性はウイルス量が0～99万個の間であることを意味している．リアルタイムPCRでは1万個以下，RT-PCRでは1,000個以下である(**表2**)．

検査を受けるときには検査法とウイルスの検出感度について確認する．

ノロウイルスによる感染症，食中毒の防止

自身が感染源とならないために，健康管理と予防に重点を置くことが重要である．

図1に示したように，ノロウイルスによる感染性胃腸炎患者の発生数に伴い，ヒト-ヒト感染による集団発生および食中毒事件が起きている．ノロウイルスによる感染性胃腸炎患者が増加すると，それは集団発生および食中毒発生の危険信号と捉え，予防対策を徹底して行わなければならない．

1．糞便・吐物の処理

糞便・吐物を直ちに処理しないと感染が拡大する．処理にはガウン，マスクとゴム手袋を着用し，自身と周りを汚染させないように注意する．処理は糞便・嘔吐物を新聞紙等で覆い，次いで市販の次亜塩素酸ナトリウム液(塩素濃度1,000 ppm)に5～10分間浸し，覆った新聞紙等を隅から中心に拭き取るようにはがす．次いで次亜塩素酸ナトリウム液(200 ppm)で拭く．塩素ガスが出るので，窓を開けて行う．

2．患者の下痢便，嘔吐物は共に液状で，手に付きやすい

ウイルスは大きさが細菌に比べ，1/30～1/100と小さく，手の皺の奥に入り込み，除去は容易でない．排便後，手が触れたところの70か所程度はウイルスに汚染され，3週間程度はその場所で感染力を維持しているので，外出先から帰ったら必ず手洗いとうがいをする．

3．手洗い

手指は石鹸(ハンドソープ剤が好ましい)を十分泡立て，温水で洗浄を10秒間以上行う．なお，ノロウイルスは容易に手から除去されないので，この操作を2回行う．特に指先，指の間，親指と小指の外側は洗わないことが多いので，この部分も確実によく洗う．高齢者，乳幼児は手洗いが十分にできないため，身の周りをより汚染するので，発病後2週間程度は手洗いを補助する．

4．その他の予防

生食する野菜，果物等(柑橘類，バナナ等)の食品は流通段階でウイルス汚染の可能性があるので，流水でよく洗浄する．

また，加熱できるもの(二枚貝の加熱用を含む)は中心温度が85℃，1分間以上を行う．ノロウイルスを殺滅させるには加熱が最も確実であるため，ノロウイルス汚染の可能性があり，加熱できるのもはすべて行う．

家族がノロウイルスに感染したときには，感染者の使用する食器等は使い捨てのものとし，できないときには使用した食器類は次亜塩素酸ナトリウム液(塩素濃度1,000 ppm)に浸し，その後水洗し，熱湯消毒する．

患者の入浴は最後とし，その後，浴槽，洗い場を次亜塩素酸ナトリウム液(塩素濃度200 ppm)消毒する．

衣類等の洗濯物は健康者とは別にし，熱湯消毒したのち，洗濯する．

排便後，汚れた手で身を整えると，自身の衣服を汚染するので，トイレ内に手洗い場を設置し，手洗い後に衣服を整える．

まとめ

ウイルスによる感染症・食中毒の防止に特別な手段はなく，自己の衛生管理，手洗いとノロウイルス汚染の危険性のあるものは加熱あるいは消毒を行うことである．

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ノロウイルス

＊国立感染症研究所感染症情報センター
西尾　治

ノロウイルスは乳幼児，高齢者における最も患者数の多い感染症であり，2006年の食中毒事件は事件数の全体の33％（504件），患者数71％（27,616名）を占め，食中毒防止からも重要視されている．ノロウイルスは感染力，物理化学的抵抗性が強く，自然界で長期間生存可能である．患者の糞便・吐物から大量にウイルスが排出され，衛生的な確保がなかなか難しく，感染症の集団発生，食中毒を容易に起こし得ることである．ノロウイルス感染防御には腸管の局所抗体が重要な役割を担うが，その抗体の持続が短く，同一・他の遺伝子型にも感染する．したがって，乳幼児から高齢者に至るまで何度でも感染・発病する．まさに，ノロウイルスは現代社会の感染症である．

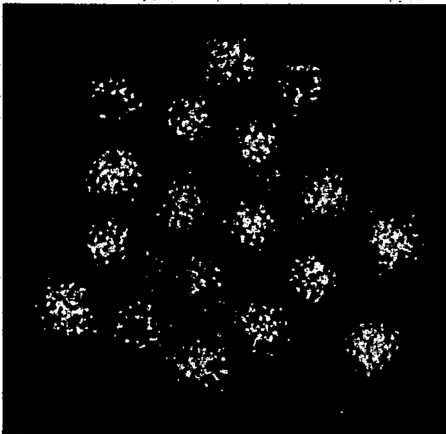

写真1　ノロウイルス電子顕微鏡写真

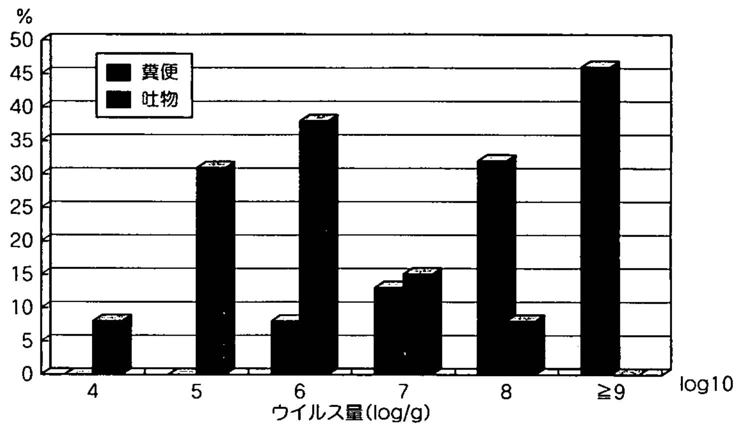

図1　急性期の患者の糞便および吐物中のウイルス量

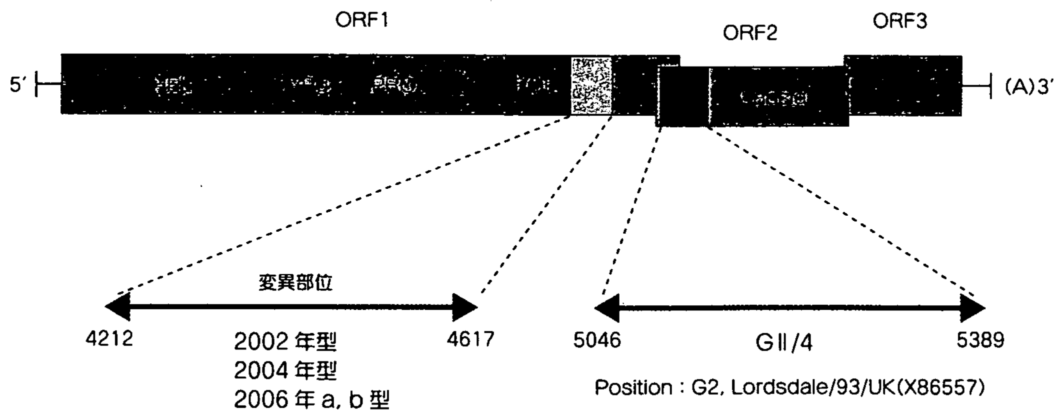

図2　ノロウイルスGII/4株の遺伝子構造と変異部位

＊〒208-0011　武蔵村山市学園4-7-1　国立感染症研究所村山庁舎

Ⅰ．ノロウイルスの歴史

ノロウイルスは1968年にアメリカ，ノーウォークで急性胃腸炎患者のふん便から電子顕微鏡で発見された[1]．形態的特徴は小さく，球形を呈し，表面に構造が見られることから小型球形ウイルス（SRSV；small round structured virus）と呼称された（写真1）．2002年8月国際ウイルス命名委員会でカリシウイルス科，ノロウイルス属と命名された．

Ⅱ．ノロウイルスの概要

本ウイルスは小さく直径30～40nm前後で球形を呈し，表面はカップ状の蛋白構造物で覆われ，その内部に長さ約7.7Kbのプラス1本鎖RNA分子ゲノムを持つ．3つの翻訳領域を有し（ORF；Open Reading Flame），ORF1はウイルス複製に必要な非構造蛋白質を，ORF2はウイルス構造蛋白であるカプシドを，ORF3は機能不明の構造蛋白質をコードしている．エンベロープはない．遺伝子型が多く，genotype（G）ⅠとⅡに大きく分けられ，GⅠは15，GⅡは18が知られている．

Ⅲ．臨床症状

例年小児科領域では180万人，昨年は360万人がノロウイルスによる感染性胃腸炎になっていると推定されている．

経口感染で，潜伏期間は通常12～72時間，主症状は嘔気，嘔吐，下痢，腹痛である．感染部位は小腸の粘膜上皮細胞で，腸管の炎症に伴う下痢症状を呈し，激しい水様性の便が数回続くこともある．胃の病変は認められないが，内容物を腸に送る運動神経の機能低下・麻痺に伴う嘔気，嘔吐の症状が見られる．嘔吐は突然，急激に強く起こり，腸内容物が逆流し，ウイルスが吐物中に入り込む．これらの症状が1～2日続いた後，治癒し後遺症も残さない．不顕性感染は30％程度と考えられている[2]．しかし高齢者，乳幼児などの抵抗力の弱いヒトでは脱水症状を起こすことがあり，時には嘔吐時に嘔吐物が気管の栓塞を，あるいは誤嚥性肺炎を起こすことがあるので注意を要する．効果のある薬剤は今のところ無く，脱水症状が強い時には補液などの対症療法が必要となる．

なお，急性期の患者の糞便には1g当たり1億個，吐物にはおよそ1g当たり100万個のウイルスが存在する（図1）[3,4]．ワクチンは開発されていない．

Ⅳ．ノロウイルスの特徴

本ウイルスの増殖系（組織培養，実験動物）が見出されていない．最近になり，腸管細胞の3D培養によりノロウイルスの感染性を知ることができる検査法が開発されたが，日常的に用いるには難しい方法で，さらに簡便な増殖方法の確立が望まれる．

ノロウイルスは感染力が強く，ウイルス粒子10個から100個で感染・発病する[2]．ノロウイルスは乾燥・液中で長期間安定であり，食品を汚染すると食中毒となる．ノロウイルスに類似のネコカリシウイルスから推測すると物理化学的抵抗性は強く，70％アルコール．塩素イオン3～6ppm，酸（pH3），アルカリ（pH10）溶液では短時間で不活化されない[5]．熱にも強く，不活化には85℃1分間の加熱が必要と考えられている．

Ⅴ．免疫

ノロウイルスの感染部位は小腸上皮細胞で，感染防御には腸管のIgA抗体が重要である．このIgA抗体は持続が短く，同じ遺伝子型の再感染が起こる．他の遺伝子型には感染防御をしないので，乳幼児から高齢者に至るまで，感染・発病を繰返すと考えられる．

Ⅵ．組織－血液型抗原と感染

ノロウイルス遺伝子型のレセプターはABO型抗原の分泌型，非分泌型（ABH型抗原が粘膜上皮細胞に発現せず，唾液中にも分泌しない），ルイス抗原との関連性が報告されている[6,7]．レセプターは遺伝子型によって異なり，ヒトは多くの遺伝子型に対するレセプターを持っているので，全てのヒトがノロウイルスに感染する．

Ⅶ．近年の流行株

2004年以降，日本のみならず世界的にノロウイルスが流行し，集団発生が多発している[8～10]．その流行株は遺伝子型GⅡ/4である．このGⅡ/4は病原性が強く，冬期のみならず他の時期に集団発生を起こすことも知られている．GⅡ/4レセプターは分泌型の血液型