Jpn. J. Infect. Dis., 62, 63-66, 2009

Short Communication

Detection of Multiple Sapovirus Genotypes and Genogroups in Oyster-Associated Outbreaks

Reiko Nakagawa-Okamoto†*, Tomoko Arita-Nishida†**, Shoichi Toda, Hirotomo Kato[1], Hiroyuki Iwata[1], Miho Akiyama[2], Osamu Nishio[2], Hirokazu Kimura[2], Mamoru Noda[3], Naokazu Takeda[4], and Tomoichiro Oka[4]

Yamaguchi Prefectural Institute of Public Health and Environment, Yamaguchi 753-0821; [1]Department of Veterinary Hygiene, Yamaguchi University, Yamaguchi 753-8515; [2]Infectious Disease Surveillance Center and [4]Department of Virology II, National Institute of Infectious Diseases, Tokyo 208-0011; and [3]National Institute of Health Sciences, Tokyo 158-8501, Japan

(Received August 4, 2008. Accepted November 17, 2008)

SUMMARY: This report describes multiple viruses in stool specimens from oyster-associated gastroenteritis. Eleven outbreaks of oyster-associated gastroenteritis were examined for enteric viruses between January 2002 and March 2006 in Japan. Multiple norovirus genotypes were detected in all outbreaks; moreover, kobuvirus, sapovirus, and astrovirus were also detected in 6, 3, and 1 of the 11 outbreaks, respectively. Notably, multiple sapovirus genogroups were detected in the stool specimens from subjects in two oyster-associated gastroenteritis outbreaks.

Viral agents of gastroenteritis affect millions of people of all ages worldwide. The major viral agents of gastroenteritis include norovirus, sapovirus, rotavirus, astrovirus, and adenovirus (1,2). Kobuvirus, which is now classified into the family *Picornaviridae*, was also recently identified as a possible pathogen for gastroenteritis (3,4). Noroviruses are the dominant cause of gastroenteritis outbreaks worldwide, and are transmitted through the ingestion of contaminated foods, through the air, and by person-to-person contact (5-7). The majority of human noroviruses can be divided into two genogroups (GI and GII) (8). Recent reports revealed sapovirus to be an important cause of gastroenteritis outbreaks (9-13), although foodborne transmission of sapovirus has not been clearly demonstrated. Sapovirus can be divided into five genogroups (GI to GV), among which GI, GII, GIV, and GV are known to be human pathogens (14,15).

The purposes of this study were to detect norovirus, sapovirus, kobuvirus, and astrovirus in stool specimens collected from subjects in oyster-associated outbreaks of gastroenteritis, and then to address the genetic diversity of norovirus and sapovirus.

Stool specimens were collected from 56 patients and 15 food handlers in 11 oyster-associated outbreaks of gastroenteritis (i.e., outbreaks in which oysters were suspected to be the cause, since all affected individuals consumed or handled oysters) between January 2002 and March 2006 in Japan. This included seven restaurants, three private homes, and a monastery (Table 1). Nucleic acids were extracted from 140 μl of a 10% (w/v) stool suspension with a QIAamp Viral RNA kit (QIAGEN K. K., Tokyo, Japan) according to the manufacturer's protocol, and reverse transcription and reverse transcription-polymerase chain reaction (RT-PCR) were performed as previously described (16). Briefly, for norovirus GI PCR, G1SKF and G1SKR primers were used; and for norovirus GII PCR, G2SKF and G2SKR primers were used (16). For sapovirus, F13, F14, R13, and R14 primers were used to amplify the 1st PCR product, whereas for the nested PCR, F22 and R2 primers were used (17). For kobuvirus, C94b and 264K primers were used, and these were designed to amplify the 3C/D junction (3). For astrovirus, PreCAP1 and 12Gr primers were used to amplify the 1st PCR product, and then Mon244 and 82b primers were used for the nested PCR (18,19). Kobuvirus- and astrovirus-positive specimens were directly sequenced, whereas norovirus and sapovirus specimens were cloned into the pCR2.1 vector (Invitrogen Japan K. K., Tokyo, Japan), and at least four clones from each specimen were sequenced. Nucleotide sequences were determined as described earlier (20). The norovirus and sapovirus sequences determined in this study were registered as EF630535-EF630617 in DDBJ.

Forty-nine of 56 (88%) stool specimens from the patients and 6 of 15 (40%) stool specimens from food handlers were positive for at least one type of virus. Interestingly, about one-third of the specimens (21 of 71 [30%]) were positive for two or more types of viruses (Table 1). Noroviruses were detected in all 11 outbreaks, including 52 of 71 (73%) stool specimens. Norovirus GI sequences were detected in 3 of 11 outbreaks, whereas we detected both norovirus GI and GII sequences in the remaining eight outbreaks. The norovirus GI sequences were separated into 10 genotypes (GI/1-5, GI/8, GI/10, and GI/13-15), while the norovirus GII sequences were separated into six genotypes (GII/3-6, GII/8, and GII/12) (Fig. 1A). Two or more genotypes of noroviruses were detected in 20 of 52 (38%) norovirus-positive specimens (Table 1).

Sapoviruses were detected in 3 of 11 outbreaks, including 5 of 71 (7%) specimens. The sapovirus sequences belonged to GI/1, GII/1, GII/2, and GII/3 (Fig. 1B). Interestingly, we detected two sapovirus genogroups in one stool specimen: SAV-H2a (GII/2) and SAV-H2b (GI/1). Kobuviruses were

*Corresponding author: Mailing address: Yamaguchi Prefectural Institute of Public Health and Environment, 2-5-67 Aoi, Yamaguchi 753-0821, Japan. Tel: +81-83-922-7630, Fax: +81-83-922-7632, E-mail: okamoto.reiko@pref.yamaguchi.lg.jp

** Present address: National Institute of Infectious Diseases, Tokyo 208-0011, Japan.

† These authors contributed equally to this study.

Table 1. Details of the outbreaks showing the setting, no. of persons with symptoms and the viruses detected

Outbreak code	M/D/Y	Setting	No. persons with symptoms	No. specimens collected	Case	Symptoms	Norovirus (genogroup/ genotype)	Sapovirus (genogroup/ genotype)	Kobuvirus	Astrovirus
1	01.23.02	Home	5	3	individual	+	H1 (GI/4)	SAV-H1 (GII/2)	–	–
	01.23.02				individual	+	H2 (GI/4)	–	–	–
	01.23.02				individual	+	H3 (GI/2)	SAV-H2a (GII/2), SAV-H2b (GI/1)	–	–
2	01.23.02	Restaurant	16	14	individual	+	I1 (GII/12)	–	–	–
	01.24.02				individual	+	–	–	+	–
	01.24.02				individual	+	I3a (GI/13), I3b (GI/4)	–	–	–
	01.24.02				individual	+	I4 (GI/13)	–	–	–
	01.24.02				individual	+	–	–	–	–
	01.24.02				individual	+	–	–	–	–
	01.24.02				individual	+	I7 (GII/12)	–	+	–
	01.24.02				individual	+	–	–	–	–
	01.24.02				individual	+	–	–	–	–
	01.24.02				food-handler	–	I10a (GI/4), I10b (GI/13)	–	–	–
	01.24.02				food-handler	–	–	–	–	–
	01.24.02				food-handler	–	–	–	–	–
	01.24.02				individual	+	I13 (GII/12)	–	–	–
	01.24.02				individual	+	I14 (GII/12)	–	–	–
3	01.30.02	Restaurant	39	2	individual	+	J1 (GI/2), J1 (GII/12)	–	–	–
	01.25.02				individual	+	J2 (GII/5)	–	–	–
4	02.26.02	Home	8	4	individual	+	K1 (GII/5)	–	–	–
	02.28.02				individual	+	K2 (GII/5)	–	–	–
	03.01.02				individual	+	K3 (GII/3)	–	–	–
	03.01.02				individual	+	K4 (GI/4)	–	–	–
5	12.25.02	Home	5	4	individual	+	L1a (GI/15), L1b (GI/8), L1a (GII/4), L1b (GII/8)	–	+	–
	12.25.02				individual	+	L2a (GI/10), L2b (GI/13), L2c (GI/4)	–	–	–
	12.25.02				individual	+	L3 (GI/14), L3 (GII/3)	SAV-L3 (GI/1)	–	–
	12.25.02				individual	+	L4 (GI/14), L4 (GII/5)	–	+	–
6	02.07.03	Restaurant	3	4	individual	+	N1 (GI/8)	–	+	–
	02.07.03				individual	+	N2 (GI/4)	SAV-N4 (GII/3)	–	–
	02.07.03				individual	+	N3 (GI/4)	SAV-N5 (GII/1)	–	–
	02.09.03				food-handler	–	–	–	+	–
7	02.16.03	Restaurant	5	3	individual	+	O1 (GI/8), O1 (GII/6)	–	+	–
	02.17.03				food-handler	–	O2a (GI/1), O2b (GI/4)	–	+	–
	02.18.03				individual	+	O3a (GII/8), O3b (GII/6)	–	–	–
8	03.01.03	Restaurant	12	14	individual	+	P1a (GI/4), P1b (GI/8)	–	+	–
	03.01.03				individual	+	P2 (GI/8), P2 (GII/3)	–	+	–
	03.01.03				individual	+	P3 (GII/4)	–	+	–
	03.01.03				individual	+	P4a (GI/2), P4b (GI/8)	–	+	–
	03.01.03				individual	+	P5 (GII/5)	–	+	+
	03.01.03				food-handler	–	–	–	–	–
	03.01.03				food-handler	–	–	–	–	–
	03.01.03				food-handler	–	–	–	+	–
	03.01.03				food-handler	–	–	–	–	–
	03.01.03				food-handler	–	P10 (GI/1)	–	+	–
	03.01.03				food-handler	–	–	–	–	–
	03.01.03				food-handler	–	–	–	–	–
	03.01.03				food-handler	–	–	–	–	–
	03.01.03				individual	+	P14 (GI/2)	–	+	–
9	12.16.04	Monastery	9	4	individual	+	R1 (GI/3)	–	+	–
	12.18.04				individual	+	R2 (GI/3)	–	+	–
	12.17.04				individual	+	R3 (GI/1)	–	+	–
	12.18.04				individual	+	–	–	–	–
10	02.14.06	Restaurant	19	15	food-handler	–	S1 (GI/8)	–	–	–
	02.14.06				individual	+	S2 (GI/8), S2 (GII/4)	–	–	–
	02.14.06				individual	+	S3 (GII/3)	–	–	–
	02.14.06				individual	+	S4 (GI/8), S4 (GII/3)	–	–	–
	02.14.06				individual	+	S5 (GII/3)	–	–	–
	02.14.06				individual	+	S6 (GI/8)	–	–	–
	02.14.06				individual	+	S7 (GI/8)	–	–	–
	02.14.06				individual	+	S8 (GI/8), S8 (GII/6)	–	–	–
	02.14.06				individual	+	S9 (GII/5)	–	–	–
	02.14.06				individual	+	S10 (GI/8), S10 (GII/3)	–	–	–
	02.14.06				individual	+	S11 (GII/4)	–	–	–
	02.14.06				individual	+	S12a (GI/14), S12b (GI/5), S12a (GII/3), S12b (GII/5)	–	–	–
	02.14.06				individual	+	–	–	–	–
	02.14.06				individual	+	S14 (GI/8)	–	–	–
	02.14.06				individual	+	–	–	–	–
11	03.07.06	Restaurant	11	4	food-handler	–	–	–	–	–
	03.07.06				individual	+	T2a (GI/8), T2b (GI/3)	–	–	–
	03.07.06				individual	+	T3 (GI/8)	–	–	–
	03.07.06				individual	+	T4 (GI/8), T4 (GII/3)	–	–	–
Total				71			52	5	19	1

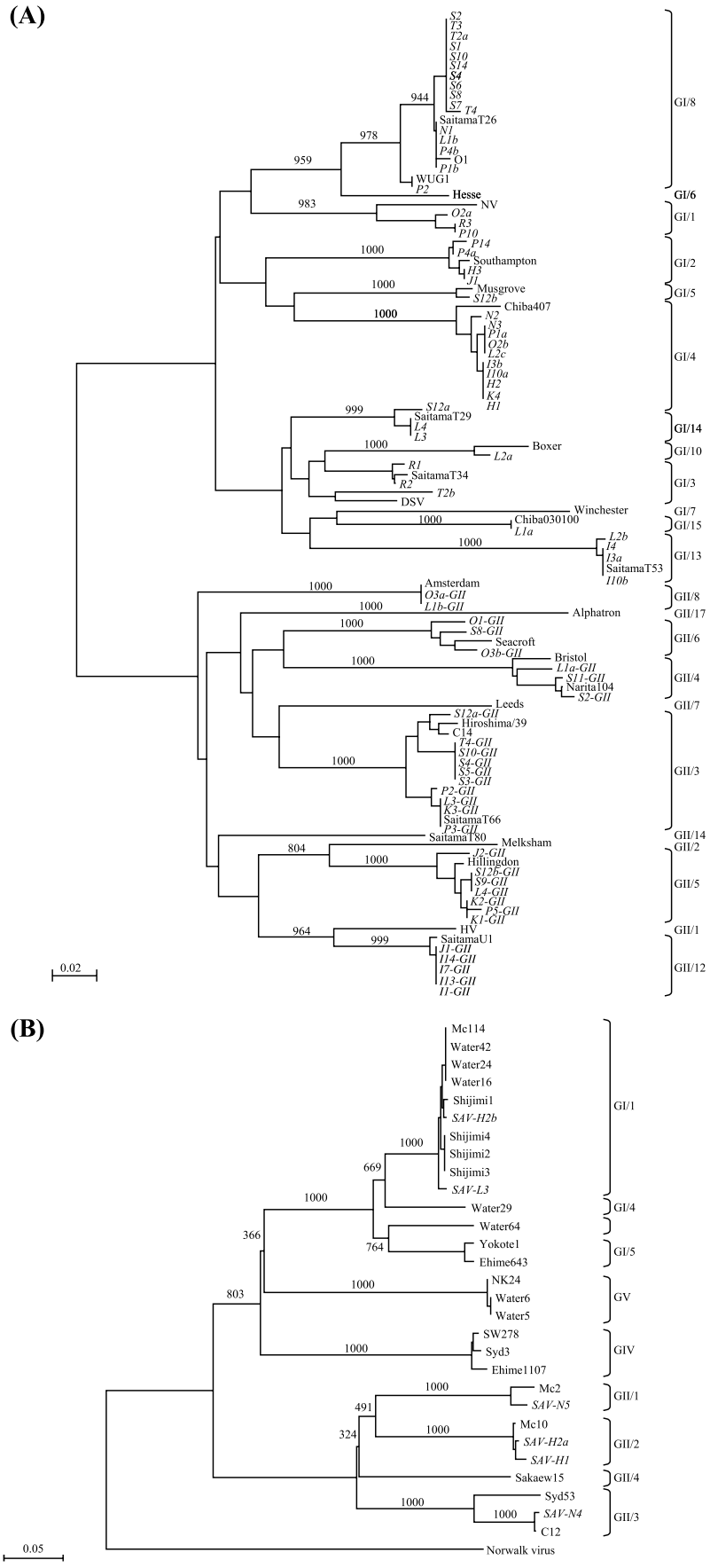

Fig. 1. Phylogenetic tree of the noroviruses (A) and sapoviruses (B) detected in this study. The trees were constructed with the partial N-terminal capsid region. The numbers on the branches indicate the bootstrap values for the clusters. Sequences and accession numbers from references (8) and (26), and Chiba030100 (AJ844469), Hiroshima/39 (AB262170), and C14 (AY845056) were used as the reference sequences.

detected in 6 of 11 outbreaks, including 19 of 71 (27%) specimens (Table 1). The kobuvirus sequences belonged to genotype A and shared greater than 98% nucleotide identity. Interestingly, 16 of 19 kobuvirus-positive specimens were also norovirus-positive, which suggests that co-contamination of these viruses in the natural environment was common. However, astrovirus was detected in only 1 of 11 outbreaks, and its nucleotide sequence was closely related to that of human serotype 4 sequences (data not shown).

In 7 of the 11 outbreaks (Outbreaks 1, 2, 5, 6, 7, 8, and 9), two or more types of viruses were detected, whereas only noroviruses were detected in the remaining four outbreaks (Outbreaks 3, 4, 10, and 11). Moreover, multiple norovirus genogroups and/or genotypes were detected in all outbreaks. It is noteworthy that we detected two sapovirus genogroups (GI/1 and GII/2) and two norovirus genotypes (GI/2 and GI/4) in one outbreak (Table 1, Outbreak 1). Although multiple norovirus genotypes were previously found, as were kobuviruses in oyster-associated outbreaks (3,4,8,21,22), this is the first report to detect multiple genotypes and genogroups of human sapoviruses in stool specimens from subjects with oyster-associated gastroenteritis. In addition, we detected two sapovirus genogroups in the same outbreak for the first time. Recently, we detected sapoviruses in the clam *Corbicula japonica* (bivalve mollusk), which is used for human consumption, and the sequences were closely related to those from patients with gastroenteritis (20). The results described in this study suggest that multiple sapovirus genotypes were concentrated in oysters, as were norovirus genotypes (23-25), which may be transmitted to humans, causing gastroenteritis. Unfortunately, no oyster samples were available for screening. The detection of sapovirus in oysters is an issue to be addressed in the future. It would also be interesting to determine whether or not the clinical symptoms of patients infected with multiple species of viruses were different from those infected with a single species of a virus.

In conclusion, sapovirus and kobuvirus were frequently detected with multiple genotypes of norovirus in stool specimens from subjects in oyster-associated outbreaks, suggesting that examination of not only norovirus but also these enteric viruses is needed in order to confirm the causative agents.

ACKNOWLEDGMENTS

This work was supported in part by a grant for Research on Emerging and Re-emerging Infectious Diseases, a grant for Research on Food Safety from the Ministry of Health, Labour and Welfare of Japan, and a grant from the Japan Health Science Foundation.

REFERENCES

1. Bon, F., Fascia, P., Dauvergne, M., et al. (1999): Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. J. Clin. Microbiol., 37, 3055-3058.
2. Sdiri-Loulizi, K., Gharbi-Khelifi, H., de Rougemont, A., et al. (2008): Acute infantile gastroenteritis associated with human enteric viruses in Tunisia. J. Clin. Microbiol., 46, 1349-1355.
3. Yamashita, T., Sugiyama, M., Tsuzuki, H., et al. (2000): Application of a reverse transcription-PCR for identification and differentiation of Aichi virus, a new member of the Picornavirus family associated with gastroenteritis in humans. J. Clin. Microbiol., 38, 2955-2961.
4. Ambert-Balay, K., Lorrot, M., Bon, F., et al. (2008): Prevalence and genetic diversity of Aichi virus strains in stool samples from community and hospitalized patients. J. Clin. Microbiol., 46, 1252-1258.
5. Gotz, H., de, J.B., Lindback, J., et al. (2002): Epidemiological investigation of a food-borne gastroenteritis outbreak caused by Norwalk-like virus in 30 day-care centres. Scand. J. Infect. Dis., 34, 115-121.
6. Marks, P.J., Vipond, I.B., Regan, F.M., et al. (2003): A school outbreak of Norwalk-like virus: evidence for airborne transmission. Epidemiol. Infect., 131, 727-736.
7. Centers for Disease, Control and Prevention (2008): Norovirus outbreak in an elementary school—District of Columbia, February 2007. Morb. Mortal. Wkly. Rep., 56, 1340-1343.
8. Kageyama, T., Shinohara, M., Uchida, K., et al. (2004): Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to *Norovirus* in Japan. J. Clin. Microbiol., 42, 2988-2995.
9. Noel, J.S., Liu, B.L., Humphrey, C.D., et al. (1997): Parkville virus: a novel genetic variant of human calicivirus in the Sapporo virus clade, associated with an outbreak of gastroenteritis in adults. J. Med. Virol., 52, 173-178.
10. Johansson, P.J., Bergentoft, K., Larsson, P.A., et al. (2005): A nosocomial sapovirus-associated outbreak of gastroenteritis in adults. Scand. J. Infect. Dis., 37, 200-204.
11. Hansman, G.S., Saito, H., Shibata, C., et al. (2007): Outbreak of gastroenteritis due to sapovirus. J. Clin. Microbiol., 45, 1347-1349.
12. Hansman, G.S., Ishida, S., Yoshizumi, S., et al. (2007): Recombinant sapovirus gastroenteritis, Japan. Emerg. Infect. Dis., 13, 786-788.
13. Wu, F.T., Oka, T., Takeda, N., et al. (2008): Acute gastroenteritis caused by GI/2 sapovirus, Taiwan, 2007. Emerg. Infect. Dis., 14, 1169-1171.
14. Farkas, T., Zhong, W.M., Jing, Y., et al. (2004): Genetic diversity among sapoviruses. Arch. Virol., 149, 1309-1323.
15. Hansman, G.S., Oka, T., Katayama, K., et al. (2007): Human sapoviruses: genetic diversity, recombination, and classification. Rev. Med. Virol., 17, 133-141.
16. Kojima, S., Kageyama, T., Fukushi, S., et al. (2002): Genogroup-specific PCR primers for detection of Norwalk-like viruses. J. Virol. Methods, 100, 107-114.
17. Okada, M., Yamashita, Y., Oseto, M., et al. (2006): The detection of human sapoviruses with universal and genogroup-specific primers. Arch. Virol., 151, 2503-2509.
18. Matsui, M., Ushijima, H., Hachiya, M., et al. (1998): Determination of serotypes of astroviruses by reverse transcription-polymerase chain reaction and homologies of the types by the sequencing of Japanese isolates. Microbiol. Immunol., 42, 539-547.
19. Yan, H., Yagyu, F., Okitsu, S., et al. (2003): Detection of norovirus (GI, GII), Sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. J. Virol. Methods, 114, 37-44.
20. Hansman, G.S., Oka, T., Okamoto, R., et al. (2007): Human sapovirus in clams, Japan. Emerg. Infect. Dis., 13, 620-622.
21. Gallimore, C.I., Cheesbrough, J.S., Lamden, K., et al. (2005): Multiple norovirus genotypes characterised from an oyster-associated outbreak of gastroenteritis. Int. J. Food Microbiol., 103, 323-330.
22. Le Guyader, F.S., Bon, F., DeMedici, D., et al. (2006): Detection of multiple noroviruses associated with an international gastroenteritis outbreak linked to oyster consumption. J. Clin. Microbiol., 44, 3878-3882.
23. Costantini, V., Loisy, F., Joens, L., et al. (2006): Human and animal enteric caliciviruses in oysters from different coastal regions of the United States. Appl. Environ. Microbiol., 72, 1800-1809.
24. Nishida, T., Kimura, H., Saitoh, M., et al. (2003): Detection, quantitation, and phylogenetic analysis of noroviruses in Japanese oysters. Appl. Environ. Microbiol., 69, 5782-5786.
25. Nishida, T., Nishio, O., Kato, M., et al. (2007): Genotyping and quantitation of noroviruses in oysters from two distinct sea areas in Japan. Microbiol. Immunol., 51, 177-184.
26. Hansman, G.S., Sano, D., Ueki, Y., et al. (2007): Sapovirus in water, Japan. Emerg. Infect. Dis., 13, 133-135.