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Occurrence of Norovirus Infections Unrelated to Norovirus Outbreaks in an Asymptomatic Food Handler Population

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Norovirus (NV) is the most common causative agent of nonbacterial gastroenteritis. Reports of surveillance of NV in facilities that reported outbreaks are frequently found in publications, but reports of that in facilities without outbreaks are not found. We investigated the molecular epidemiology of NV isolates derived from asymptomatic food handlers working at a nonoutbreak food catering facility in Hokkaido, Japan, from February to March in 2005 and January to February in 2006 by RNA polymerase gene sequencing. Approximately 12% (20/159) of the samples were positive for genogroup II (GII; 10.1% in 2005 and 14.2% in 2006). The GII genotypes were not detected. The data from the phylogenetic analysis indicated that, among the 20 strains detected, 13 strains were GII genotype 2 (GII/2), two were GII/3, three were GII/8, and two were GII/12, GII/4, which has been found most frequently in recent outbreaks worldwide, including Japan, was not detected. We found that one individual was coinfected with two genotypes, GII/2 and GII/12. This is the first report of the detection of NV genotypes in asymptomatic food handlers working at a nonoutbreak facility. The excretion of NV from healthy individuals may be an infection source of NV outbreaks as well as other food-borne diseases.

Norovirus (NV) is now recognized as the leading cause of nonbacterial acute gastroenteritis, causing numerous outbreaks worldwide (4, 10). NV is a member of the Caliciviridae family. According to the sequence analysis of genes encoding the RNA-dependent RNA polymerase or the capsid protein, NV is classified into five genogroups; genogroup I (GI) is further subdivided into 15 genotypes, and GII is subdivided into 18 genotypes (13). Molecular epidemiological studies suggest that a number of NV genotypes circulate in different facilities, whereas GII genotype 4 (GII/4) has been the most prevalent genotype in outbreaks worldwide, including Japan (5, 6, 14, 16).

NV occasionally causes outbreaks in facilities such as restaurants, schools, day-care centers, hospitals, nursing homes, and cruise ships (1, 7, 8, 12, 15). Factors such as a very low infectious dose, the absence of long-lasting immunity, the stability of the virus in the environment, and its transmission by a variety of routes contribute to the high impact of NV outbreaks (11).

NV outbreaks peak in the winter in Japan, and NV is therefore referred to as “the winter vomiting disease.” In Japan, oyster-associated gastroenteritis has also caused a number of outbreaks (9). However, transmission of NV by the fecal-oral route through person-to-person contact has been a major cause of outbreaks in various facilities in recent years (1). Approximately 30% of food-borne NV outbreaks in the United States are linked to ill food handlers (17). Ozawa et al. reported that the frequency of NV detection was 19% in outbreak facilities in Japan and that 73% of symptomatic food handlers and 7% of asymptomatic food handlers were positive for NV (14). They suggested that asymptomatic infections are common and contribute to the spread of the infection in areas of outbreak.

There have been many reports regarding the monitoring of NV in facilities with outbreaks. However, little information is available about circulating viral strains in asymptomatic individuals in facilities without NV outbreaks. In this study, we investigated NV epidemiology in asymptomatic food handlers working at a food catering facility without any cases of acute gastroenteritis in Sapporo City, Hokkaido, Japan. This paper describes the first detection of asymptomatic human infection by NV in a food catering facility without reported NV outbreaks.

MATERIALS AND METHODS

Fecal samples (n = 159) were collected from asymptomatic food handlers in a food catering facility of a hotel without any reported cases of acute gastroenteritis in Sapporo City, Hokkaido, Japan. These samples were collected from donors, diagnosed as being without gastroenteritis, at a regular medical checkup. Informed consent was obtained from all donors. The collection periods were from February to March in 2005 (89 samples) and January to February in 2006 (70 samples). These months coincide with the peak outbreak periods in Japan. The stool suspensions (10% [vol/vol] in phosphate-buffered saline, pH 7.0) were centrifuged at 4,000 × g for 20 min. Viral RNA was extracted from the supernatant using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Part of the RNA-dependent RNA polymerase gene was amplified by nested PCR using the primers described by Kawamoto et al. (10). First-step reverse transcription-PCR (RT-PCR) was performed using the P1/P3 or the NV51/NV62 and SM82 primer set with the One Step RT-PCR kit (Qiagen). The second-step PCR was performed using the RT-PCR products and the P1/P2 or Y1/Y2 primer set with the HotStarTaq Mastermix kit (Qiagen). The resulting PCR products were directly sequenced using the ABI Prism Big Dye Terminator cycle sequencing kit and the ABI Prism 310 DNA sequencer (Ap-
plied Bio systems, Foster City, CA). The resulting 176-bp sequences were then subjected to phylogenetic analysis using Genetix-Mac, version 10 (Software Development, Tokyo, Japan). Phylogenetic reconstructions were carried out using the UPGMA (unweighted-pair group method using average linkages) with the Kimura two-parameter model. Statistical confidence for the tree was assessed by bootstrap analysis (1,000 replicates). Published Caliciviridae sequences were included for alignments and phylogenetic analysis. GenBank strains are NV (Cambridge virus [X86557], Lordsdale virus [X81879], Norwalk virus [M87661], and Sapovirus [U02030]) and Sapovirus (Sapporo virus [X86560]).

**RESULTS**

Among the 159 specimens examined, 20 specimens (12.6%) were positive for NV, as indicated by NV-specific nested PCR (Table 1). The positive PCR products were confirmed by the sequence analysis to contain NV partial genomic sequences. In one specimen, the amplified sequence did not match any known NV sequences. Nineteen specimens (11.9%) had NV-specific sequences, and this proportion of NV-positive asymptomatic individuals (10.1% in 2005 and 14.2% in 2006) was similar in samples from the two years. These results indicate that a significant number of asymptomatic, NV-infected individuals may be present in nonoutbreak conditions.

Sequences for the 176-bp region of the RNA polymerase gene were obtained from the 20 PCR products. In one specimen, PCR products were amplified from both primer sets. The sequences were compared with those of strains obtained from the GenBank database (Table 1; Fig. 1). All of the sequence-positive samples belonged to the NV GII genotype. Among the 20 samples, 13 were GII/2, 2 were GII/3, 3 were GII/8, and 2 were GII/12. One specimen contained two genotypes, GII/2 and GII/12. Sequences of all strains belonging to a particular genogroup were identical.

In the sample population, 56 individuals were assayed for NV in both 2005 and 2006. Among these individuals, only one person was positive for NV in both 2005 and 2006; however, the genotypes were different (GII/8 and GII/2, respectively). The infection rate of asymptomatic individuals in nonoutbreak environments in this study and that in outbreak cases in Japan were comparable. This result suggests that population normally contains asymptomatic carriers of NV regardless of the occurrence of NV outbreaks.

Several genotypes belonging to GII were detected in this study. Among these, GII/2 appeared to be the dominant genotype. We also examined the outbreak cases in Sapporo City, Hokkaido, Japan, in 2005 (five cases) and 2006 (five cases), which had been referred to the Sapporo City Institute of Public Health. These outbreaks occurred in the same city and in the same time frame as this study. Among these cases, six genotypes were determined: one case of GII/3 and one of GII/9 in 2005 and one case of GII/3, one of GII/6, and two of GII/4 in 2006 (data not shown). These results indicate that several strains of NV caused outbreaks in Sapporo City in 2005 and 2006. Recently, GII/4 and its variants have been the most prevalent in worldwide outbreaks (5, 6, 16). Ozawa et al. reported that GII/4 was the predominant genotype, since that genotype was found in 35% of outbreak cases and 46% of sporadic cases in Japan in 2005 and 2006 (14). Additionally, asymptomatic food handlers, at the NV outbreak facilities, were also infected with GII/4. Asymptomatic individuals also excreted viral loads similar to those of symptomatic individuals (14). Amar et al. also indicated the occurrence of viral infection of intestinal disease in case patients as well as in asymptomatic individuals; however, quantification of the viral load may indicate a difference between symptomatic and asymptomatic individuals (2). Susceptibility to a particular genotype could differ among individuals, perhaps due to differences in innate and acquired immunities against NV. In the facility that we examined, NV outbreaks did not occur even though NV carriers were present. Several factors may contribute to this effect, such as (i) pathogenicity of the virus, (ii) the number of individuals infected and the amounts of virus excreted, (iii) protective immune status of those exposed, and (iv) shutoff of infection routes.

One individual in this study was coinfected with two genotypes of NV, GII/2 and GII/12. The mixed infection could lead to viral recombination and generation of a new NV strain with altered virulence properties (3).

**DISCUSSION**

In this study, NV RNA was detected in 11.9% of asymptomatic individuals tested in a group without NV outbreaks. In other studies, asymptomatic carriers were found to make up 7% of the food handlers associated with outbreaks in Japan (14) and 26% and 33% of staff and patients, respectively, involved in a hospital outbreak in the United Kingdom (4).
FIG. 1. Phylogenetic tree of the NV GII sequences detected in this study. The number (n) of each strain detected in each year of this study is underlined. The tree was constructed using NV partial sequences of the RNA-dependent RNA polymerase gene, using the Sapporo virus as an outgroup.
in healthy individuals. The excretion of NV from an asymptomatic population (e.g., food handlers) may cause outbreaks of NV as well as many other food-borne diseases.

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