Epidemiological Report

Multiple Outbreaks of Gastroenteritis Due to a Single Strain of Genotype GII/4 Norovirus in Kobe, Japan, 2006: Risk Factors for Norovirus Spread in Health Care Settings

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SUMMARY: A large number of gastroenteritis outbreaks due to norovirus GII/4 strain and its variants occurred during November and December 2006 in Kobe, Japan. Of the 118 outbreaks, 6 were foodborne and 112 were caused by person-to-person transmission in healthcare settings such as nursing homes and hospitals. The distribution of norovirus outbreaks in healthcare settings was skewed, particularly in the south coastal area. Moreover, several outbreaks occurred within 1 km² in various areas. Outbreaks in neighboring settings, especially within 1 km, and travel from the sources of outbreaks were risk factors for the spread of the norovirus. The use of ineffective disinfectants such as alcohol and benzalkonium chloride might also have helped to spread the infection.

INTRODUCTION

Noroviruses are recognized as a worldwide cause of acute nonbacterial gastroenteritis. Norovirus infection is common in all age groups and is characterized by a low infectious dose and high attack rate (1). The virus is transmitted predominantly through ingestion of contaminated food as well as person-to-person by the fecal-oral route, airborne transmission, and contact with contaminated surfaces (2-4).

Foodborne outbreaks are frequently associated with the consumption of fecally contaminated shellfish, particularly oysters. Contamination via infected food handlers has become a common source of large outbreaks (5-7). In addition, outbreaks caused by person-to-person transmission are frequent in settings where close personal contact is routine such as nursing homes, hospitals, and nursery schools.

Norovirus genetically comprises 5 distinct genogroups, but most human noroviruses can be classified into genogroups I (GI) and GII, which can be further subclassified into 15 and 18 genotypes, respectively (8). Several outbreaks of gastroenteritis due to noroviruses, particularly genotype GII/4, have recently occurred in England, Germany, Sweden, the Netherlands, Ireland, the United States, Australia, Japan, and elsewhere, indicating a global spread of the genotype (8-15).

In November and December 2006, an unusually large number of gastroenteritis outbreaks due to norovirus GII/4 occurred in various parts of Japan (16-18). The number of outbreaks of gastroenteritis during that winter season in Kobe was more than eightfold higher than that of the previous year. We describe herein the features of the outbreaks in Kobe and the risk factors for norovirus infection derived from an epidemiological study of the outbreaks and molecular analysis of the causative agents.

MATERIALS AND METHODS

Study area and outbreaks: Kobe City is located in the center of Japan and is accessible by land, sea, and air. It covers an area of 552 km² (36 km from east to west and 30 km north to south) and has a population of 1,530,847 as of 1 January 2008. It comprises 9 wards with populations ranging from 102,657 to 248,754 per ward.

Outbreaks of gastroenteritis in Japan are reported to local government public health centers by order of the Ministry of Health, Labour and Welfare. During November and December 2006, 118 outbreaks of norovirus gastroenteritis at 67 nursing homes for the elderly, 30 hospitals, 7 nursery schools, 6 institutions for the mentally handicapped, 5 restaurants, 2 institutions for homeless or maltreated children, and 1 elementary school were reported to Kobe City Public Health Center. Outbreaks in which the epidemic curve comprised a single peak were considered as foodborne. Others were treated as person-to-person transmission. When the disinfectants used were recorded in the reports, they were classified into 2 groups: effective against norovirus or not.

Virus detection: During November and December 2006, 505 stool specimens were collected from guests and staff at the 5 affected restaurants (n = 284) and from residents, inpatients, and staff of 39 healthcare institutions (33 nursing homes and 6 hospitals; n = 221). Stool samples were suspended in ninefold volumes of phosphate-buffered saline (PBS) and separated by centrifugation at 10,000 × g for 30 min. Nucleic acids were extracted from the supernatants using the E.Z.N.A. viral RNA kit (Omega Bio-tek, Leicestershire, UK) according to the manufacturer’s instructions. Samples of RNA were diluted 10-fold to avoid the effects of PCR inhibitors and screened by one-step real-time RT-PCR (Applied Biosystems, Foster City, Calif., USA) using the primers COG1F and COG1R with the probes RING1(a)-TP and RING1(b)-TP for the GI genogroup, and the primers COG2F, ALPF, and COG2R with the RING2-TP probe for the GII genogroup (19). The PCR proceeded in 5 μ1 of RNA templates and 20-μ1 reaction mixtures containing RNase inhibitor, reverse transcriptase, DNA polymerase, dNTPs, primers, and probes. The final concentrations of primers and probes were 400 and 250 nM, respectively. The amplification conditions were as follows: 30 min at 48°C, 10 min at 95°C, 50 cycles of 95°C for 15 s and 56°C for 1 min. GI-positive samples were further analyzed by one-step real-time RT-PCR using the novel...
primers, GII.4-F (5’-AAYAATGAGGTATGGCTYTGGAG-3’) and GII.4-R (5’-GGYACRGGYGCYGCAA-3’), and the probe, GII.4-P (5’-FAM-CCCCTTGGYTGGYGCYCGC-TAMRA-3’), for the GII.4 genotype under the same amplification conditions.

Sequencing: The nucleotide sequence (2,383-nt) of the 3’ terminus of RNA polymerase and capsid regions of a typical strain, Kobe034/2006/JP, were deposited in the GenBank/EMBL/DDBJ databases (accession no. AB291542). The 3’ terminus of RNA polymerase and the 5’ terminus of capsid regions (387-nt) of 93 samples that were norovirus-positive were amplified by one-step RT-PCR (Qiagen, Valencia, Calif., USA) using the primers COG2F and G2-SKR (19,20), and were directly sequenced from both sides using the same primers. The sequences from which primer regions were excluded (338-nt) were compared.

RESULTS

Features of outbreaks: The outbreaks were more frequent in 2006 than in either 2005 or 2007 (Fig. 1). The number of outbreaks in 2006 increased remarkably from week 47 to week 49, and decreased thereafter. Of the 118 outbreaks in 2006, epidemic curves showed that 6 were foodborne, 5 originated in restaurants, and 1 was due to meals provided at a nursing home for the elderly. No shellfish, including oysters, were involved in any of the foodborne outbreaks. Of the 118 outbreaks, 112 seemed to be due to person-to-person transmission because none of the epidemic curves comprised a single peak.

Ten gastroenteritis outbreaks occurred in 6 city wards during weeks 44 and 45, and then gradually spread to neighboring areas (Fig. 2). As of week 48, no outbreaks of gastroenteritis occurred in 1 city ward or on 2 artificial islands, but gastroenteritis rapidly spread thereafter throughout Kobe City. However, the locations of the gastroenteritis outbreak were skewed, particularly in the south coastal area. Several outbreaks occurred within 1 km of each other in various areas. In contrast, scattered outbreaks were unusual. Only 14 (12.9%) settings were >1 km from the locations of other outbreaks.

Of the 28 outbreaks during weeks 44 to 46 in 2006 in which disinfectant use was reported, 10 (35.7%) of the facilities used alcohol, benzalkonium chloride, and/or chlorhexidine gluconate, none of which can inactivate norovirus (21,22). Although the remaining 18 settings used sodium hypochlorite, whether or not the concentrations were high enough to inactivate norovirus remains unclear. During weeks 49 to 52, only 4 (8.7%) of the 46 facilities used ineffective disinfectants.

Causative agents: Of the 118 outbreaks of gastroenteritis in November and December 2006, laboratory investigations confirmed that 95 of them were caused by noroviruses. The other 23 outbreaks were not analyzed. Of the 44 outbreaks studied at our laboratory, all causative agents belonged to the norovirus GII/4 genotype. Neither GI nor GII genotypes, except for GII/4, were detected, although various genotypes were detected from gastroenteritis outbreaks before 2006 in Japan (8,23,24).

The 338-nt sequences of noroviruses obtained from 31 (70.5%) of the 44 outbreaks (21 nursing homes, 5 hospitals, and 5 restaurants) were identical, with a few exceptions. Among the 31 outbreaks, substitutions at 338-nt were found in 3 patients (1-nt, 2 patients; 2-nt, 1 patient). These mutations likely occurred within the individual patients because the sequences of noroviruses obtained from other patients during the same outbreaks were identical to that of the typical strain. Of the 6 food-related outbreaks, noroviruses with identical sequences were detected from food handlers in 4 restaurants, suggesting that contamination was spread via the hands of the food handlers.

The remaining 13 outbreaks were caused by similar strains with mutations of ≤4-nt at 338-nt (1-nt, 6 facilities; 2-nt, 2 facilities; 3-nt, 4 facilities; 4-nt, 1 facility). The norovirus sequences at each outbreak were identical in several samples, with 1 exception (1-nt substitution). Two strains with 1-nt and 3-nt substitutions at fixed positions were detected from outbreaks at 2 and 3 locations, respectively.

DISCUSSION

A single norovirus GII/4 strain caused a large number of gastroenteritis outbreaks in Kobe during November and December 2006. Among the 44 outbreaks studied, 13 (29.5%) seemed to be caused by norovirus variants, as mutations were detected only at 4-nt or less in 338-nt of the norovirus GII capsid region, which had conserved overlapping 17-nt and approximate 300-nt of the average mutation rate (19).

Detection of mutations is a potential indicator of transmission routes (10), but only 2 strains with 1- and 3-nt substitutions at fixed positions were detected from outbreaks at 2 and 3 locations, respectively. Two of the locations at which outbreaks were caused by a strain with a 1-nt substitution were neighboring city wards. However, whether transmission was responsible or a mutation occurred at the same position was unclear because the mutation was limited and no relationship was identified between the 2 settings. In contrast, the outbreaks that were caused by a strain with 3-nt substitutions seemed to be transmitted at 2 of 3 healthcare facilities that were under corporate administration, indicating that the staff might have been involved in the transmission. Thus, travel from the source of an outbreak might be a risk factor for norovirus spread.

Transmission routes could not be determined in the present study based on mutation analyses, with only 1 exception, because the 338-nt sequences of noroviruses obtained from 31 (70.5%) of the 44 outbreaks were identical. The distribution of locations of gastroenteritis outbreaks was skewed in small areas (Fig. 2). Outbreaks in neighboring locations, particularly within 1 km, might be a risk factor for norovirus infection because person-to-person contact, airborne transmission, and contact with contaminated surfaces are presum-
ably the predominant routes of transmission (1,2,4).

The reasons why a single GII/4 strain caused such a large number of outbreaks remain obscure. High circulation rates, a low infective dose, and environmental stability might be associated (4,25). The use of ineffective disinfectants such as alcohol and benzalkonium chloride might also have helped to spread the infection. A BLAST search of the DDBJ database showed that the strain was more similar (99.4% identities) to those that caused outbreaks in Hong Kong and the Netherlands in 2006, than to those detected in Japan before 2006 (<97.7% identity), suggesting that a specific strain has spread worldwide. The transmission route of the strain found in Kobe remains unknown. Future studies will be directed towards understanding the cause of the worldwide spread of the GII/4 strains.

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