JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1093–1098 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1093–1098.2005

Multiprefectural Spread of Gastroenteritis Outbreaks Attributable to a Single Genogroup II Norovirus Strain from a Tourist Restaurant in Nagasaki, Japan

Yoichi Hirakata,[1]* Kokichi Arisawa,[2]† Osamu Nishio,[3] and Osamu Nakagomi[2]

Department of Laboratory Medicine, Nagasaki University School of Medicine and Dentistry,[1] and Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences,[2] Nagasaki, and Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo,[3] Japan

Received 28 June 2004/Returned for modification 19 August 2004/Accepted 4 November 2004

A series of gastroenteritis outbreaks caused by noroviruses (NVs) among tourist groups from several prefectures was associated with eating a lunch prepared by a restaurant in Nagasaki City, Japan, on 18 and 19 November 2003. A retrospective cohort study was performed to estimate the magnitude of the outbreak and identify the source of infection. Epidemiological information was obtained through the local public health centers in the areas where the illness occurred. Stool and vomit specimens and food and environmental samples were analyzed by reverse transcription-PCR with genogroup-specific primers. Positive samples were sequenced and analyzed phylogenetically. Of 1,492 tourists who ate a lunch prepared by the restaurant during the 2-day period, 660 (44.2%) developed illness, with an average incubation time of 31.2 h. Whereas NVs were not detected in any food samples, identical sequences most closely related to the Mexico genotype of genogroup II NV were found in specimens from case patients, restaurant staff, and the kitchen table. Food handlers were concluded to be the source of the outbreak as a result of the contamination of several meals. The series of outbreaks described here exemplifies the role of tourism as a contemporary way to distribute a single infectious agent to multiple and geographically remote areas.

Norovirus, a genus within the family *Caliciviridae*, has emerged as an important cause of food- and waterborne gastroenteritis outbreaks in industrialized countries (6, 8, 20). Noroviruses (NVs) are responsible for 78.5% of all nonbacterial outbreaks of gastroenteritis reported from 1995 to 2000 in Europe (21). They accounted for an estimated 6 to 14, 11 to 18, and 20% of infectious intestinal diseases in England and Wales (3, 7, 28), The Netherlands (4, 17), and Finland (27), respectively. It was reported that 96% of 90 outbreaks of nonbacterial gastroenteritis were caused by NVs (6), and it is estimated that NVs cause 23 million illnesses each year (22) in the United States. In Japan, NVs accounted for 28% of cases of food poisoning from all causes and 99% of cases from purely viral sources (24).

NVs can be classified into five genogroups, genogroups GI to GV; the three genogroups GI (prototype strain, Norwalk virus), GII (prototype strain, Snow Mountain virus), and GIV have been found in humans (1, 23, 29, 31). Reverse transcription-PCR (RT-PCR) has become a favored method for detection and classification of NVs and has extensively been used as a tool in investigations of acute gastroenteritis outbreaks (9, 13, 30, 33). Little has been reported about the genotype distribution of NVs in Japan. The GII Lordsdale genotype (GII/4) has been predominant since 1996, and the GII Mexico genotype (GII/3) suddenly appeared and spread during the 1999-2000 season in Osaka City, Japan (11). In another study, various genotypes of NVs were found in Kyushu, Japan, from 1988 to 1993, and the GII Mexico genotype was dominant in 1989 (26). In Japan, raw oysters are the primary source of transmission in small outbreaks, whereas school lunches and catered meals, banquet halls, and hospitals are most often implicated as the vehicles and settings of transmission in large outbreaks (those involving >50 patients) (10). In terms of the number of patients involved in NV gastroenteritis outbreaks in Japan, the largest one (3,236 schoolchildren) occurred in nine elementary schools in 1989 following consumption of a school lunch prepared by a lunch preparation center in which one food handler had gastroenteritis (15).

In this article we describe the investigation into a series of gastroenteritis outbreaks that occurred among tourists who had a lunch prepared by a single tourist restaurant and that were attributed to a single strain of NV.

MATERIALS AND METHODS

Outbreak description. Multiple outbreaks of acute gastroenteritis occurred among the tourists from several prefectures who visited Nagasaki City, Japan, and who had a lunch prepared by a tourist restaurant (restaurant J) in November 2003. Nagasaki City is located in the western part of the island of Kyushu, has a population of 420,000, and is visited by more than 5 million tourists a year. On 19 November, the Public Health Authority in Nagasaki City initially received two independent calls that students and teachers from schools in different prefectures who had visited Nagasaki City on a school excursion the day before had gastrointestinal symptoms, such as nausea, vomiting, diarrhea, abdominal pain, and fever. It turned out that the members of these tourist groups had lunch at restaurant J or ate box lunches prepared by that restaurant.

Thus, the Public Health Authority immediately suspended the business of restaurant J, as it was the suspected origin of the food-poisoning outbreak. Gastroenteritis cases continued to occur among tourists who had lunch prepared

* Corresponding author. Mailing address: Department of Laboratory Medicine, Nagasaki University School of Medicine and Dentistry, Nagasaki 852-8501, Japan. Phone: 81958497418. Fax: 81958497257. E-mail: hirakata@net.nagasaki-u.ac.jp.

† Present address: Department of Preventive Medicine, The University of Tokushima Graduate School Institute of Health Biosciences, Tokushima. Japan.

TABLE 1. Characteristics, attack rates, and incubation times for the groups that ate at restaurant J on 18 November

Group	Type of group	Time of visit	No. of tourists	No. of patients	Attack rate (%)	Incubation time (h)[a]
A	Junior high school excursion from Kagoshima	Box lunches	32	25	78.1	27.8 ± 7.1
B	Adults from inside Nagasaki Prefecture	11:30	26	2	7.6	33.5 ± 2.8
C	Elementary school excursion from Fukuoka	11:40	103	73	70.9	33.0 ± 8.6
D	Adults from Osaka	12:30	17	5	29.4	30.1 ± 10.6
E1	High school excursion from Aichi	12:30	415	322	77.6	29.8 ± 10.3
Total			593	427	72.0	30.1 ± 10.0

[a] Values are means ± standard deviations.

by restaurant J on 19 November. Restaurant J was open for tourist groups only on a subscription basis and had a kitchen staff of 10, including 2 cooks, at the time of the event. Single parties of less than 30 tourists each visited restaurant J each day between 15 and 17 November. However, a total of 11 groups ate food from restaurant J on 18 and 19 November; 593 tourists among 5 groups (groups A to E1) ate food from the restaurant on 18 November, and 931 tourists among 7 groups (groups A, E2, and F to J) ate food from the restaurant on 19 November (Tables 1 and 2).

Epidemiological investigation. A retrospective cohort study of the 11 groups that ate food from restaurant J on 18 and 19 November was conducted. Since the case patients became ill at home or during their trip after they left Nagasaki City, information was obtained through the local public health centers in the administrative regions where the case patients affected by gastroenteritis lived. The questionnaires, standardized by the Ministry of Health, Labour and Welfare, were used to obtain information about the sex and age of each of the patients, the time of onset and nature of their symptoms, and what foods they ate.

A case was defined as the development of at least two of the following symptoms in any tourist who had eaten food from restaurant J on 18 and 19 November: nausea, vomiting, diarrhea, abdominal pain, and fever.

The restaurant employees were interviewed in detail. We investigated the hotels and other restaurants in Nagasaki City that the 11 groups used during their trips. We also interviewed other tourist groups that visited Nagasaki City during the same period but that did not consume food from restaurant J. Information on the secondary cases was gathered through the local public health centers.

Environmental investigation. The facility was inspected by the Food Hygiene Section of the Nagasaki City Health Department on 20 and 21 November. The storage conditions of the meals and bulk food items were investigated, and several food samples were taken. A total of 29 smears of environmental samples were also taken from the restaurant, including the kitchen and the washroom. Stool specimens from all kitchen staff were submitted on 21 and 22 November.

Microbiological investigation. The vomit and stool specimens from the case patients were cultured for bacterial enteropathogens, including *Salmonella*; *Shigella*; enteropathogenic *Escherichia coli*, including *E. coli* O157; *Campylobacter*; *Yersinia*; *Vibrio*; *Aeromonas*; *Plesiomonas*; *Staphylococcus aureus*; *Clostridium perfringens*; and *Bacillus cereus*. Approved standard laboratory methods were used for all bacteriological investigations.

RNA extraction, RT-PCR, and sequencing. Samples and specimens were examined for NVs by RT-PCR, as described elsewhere (24, 33). Genogroup-specific primers were used to amplify the partial capsid region of NVs by RT-PCR (16, 24), as follows: primers COG1F and G1-SKR and primers COG2F and G2-SKR for amplification of the GI and GII NVs, respectively. For some samples, a nested PCR was performed with primers G1-SKF and G1-SKR (GI) and with primers G2-SKF and G2-SKR (GII). We also quantified the NV capsid genes for some PCR-positive samples by using a real-time PCR, as described previously (13, 24). The detection limits were 10^1 and 10^2 copies for the food and environmental samples and the clinical specimens, respectively (data not shown).

The capsid sequences were aligned, and the nucleotide sequence identities were analyzed with GENETYX-MAC software (version 11.0). The nucleotide sequences were compared with those of reference strains of NVs obtained from GenBank for the phylogenetic analysis, as described previously (14).

Statistical analysis. Data are presented as means (standard deviations and ranges) or as counts or proportions. Student's *t* test was used to compare the means between the two groups. The chi-square test was used to assess the statistical significance of the associations among variables. We calculated odds ratios (ORs) using Woolf's procedure and multivariate ORs using multiple logistic regression analysis (SAS, version 8.2) for each group and Mantel-Haenszel ORs for all subjects together, with 95% confidence intervals (CIs), to assess whether there was any association between illness and an individual meal, food, or food item. A P value less than 0.05 was considered significant.

Nucleotide sequence accession number. The NV capsid sequence data have been submitted to GenBank and assigned accession number AY590117.

RESULTS

Epidemiological investigation. All 10 tourist groups in which gastroenteritis cases occurred had eaten lunch at restaurant J or ate box lunches prepared by this restaurant. By contrast, there were no reports of illness among 44 tourist groups (2,371 persons) who visited Nagasaki City during the same period but who did not dine at restaurant J ($P < 0.001$). No hotels or restaurants, other than restaurant J, where the 10 groups stayed or visited reported the occurrence of gastroenteritis. Consequently, restaurant J was concluded to be the causative facility of the outbreak.

Tables 1 and 2 show the times and the dates when the

TABLE 2. Characteristics, attack rates, and incubation times for the groups that ate at restaurant J on 19 November

Group	Type of group	Time of visit	No. of tourists	No. of patients	Attack rate (%)	Incubation time (h)[b]
F	High school excursion from Hokkaido	Box lunches	163	97	59.5	34.2 ± 10.4
G	Elementary school excursion from Kumamoto	11:00	145	37	25.5	28.3 ± 12.7
E2	High school excursion from Aichi	11:45	294	63	21.4	39.3 ± 16.4
H	Elementary school excursion from Kumamoto	12:10	169	35	20.7	24.5 ± 15.0
I	Adults from Gunma	12:10	15	0	0.0	
A	Junior high school excursion from Kagoshima	12:40	32	25[a]	78.1[a]	27.8 ± 7.1[a]
J	Junior high school excursion from Kagoshima	12:50	113	1	0.9	25.8
Total			931	233	25.9	33.1 ± 14.2

[a] The case patients were thought to be infected on the first day because of the incubation period.
[b] Values are means ± standard deviations.

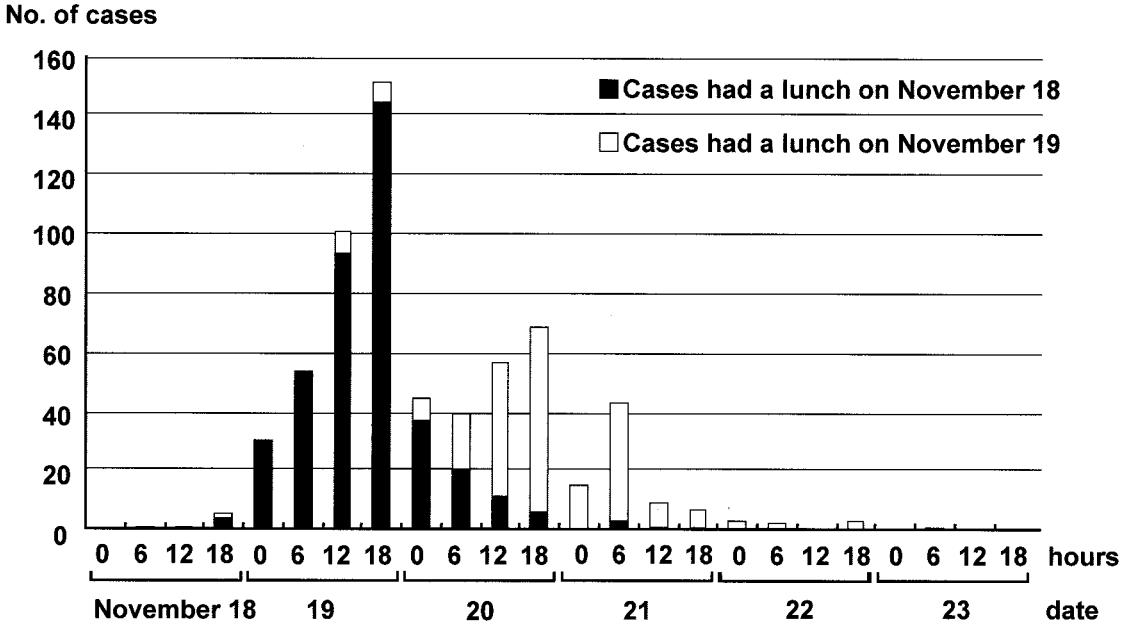

FIG. 1. Epidemic curve of cases, by hours and dates of onset of symptoms. The *x* axis presents the times (in hours) and the days when the onset of symptoms occurred.

tourists visited Restaurant J, the type of tour, the numbers of tourists and cases, the attack rates, and the incubation times for each group. Group A consumed meals from restaurant J on both 18 and 19 November. Groups E1 and E2 belonged to the same school and visited restaurant J on 18 and 19 November, respectively. The questionnaires were received from 97.3% of the tourists (35.3 to 100% for each group). Most groups responded very well (96.1 to 100%), whereas group D, which consisted of adult individuals only, responded poorly (35.3%).

Of the 1,492 tourists who used restaurant J, 660 developed illnesses that met the case definition. Thus, the overall attack rate was 44.2%. The mean age was 17.0 ± 8.4 years (age range, 11 to 74 years); and 90.6% of the cases occurred among students in elementary, junior high, and high schools (age range, 11 to 18 years). There was no sex-related difference in the attack rates, which were 46.8% for males and 44.7% for females ($P = 0.64$). The attack rates were invariably greater than 70% for the students who had lunch at restaurant J on 18 November, while they gradually decreased for those who had lunch there on the next day. There was a significant difference ($P < 0.001$) in the attack rates between the groups that ate lunch on the first day (72.0%) and the next days (25.9%) of the outbreak. The attack rate was low in groups B and D, and there were no illness in group I; although the amounts and types of foods consumed did not differ, all these groups were commonly adult tourist parties. The symptoms most commonly reported by case patients were nausea (87.0%), vomiting (71.8%; 4.0 times a day, on average), abdominal pain (69.5%), fever (68.6%), and diarrhea (54.4%; 3.1 times a day).

The epidemic curve shows two peaks (Fig. 1), but each peak represents a cluster of cases among those who ate food from the restaurant on either 18 or 19 November and has a pattern characteristic of a single-exposure, common-vehicle outbreak. The mean incubation time was 31.2 ± 11.7 h, and there was no difference in the incubation times between the tourists who consumed food from restaurant J on the first day (30.1 ± 10.1 h) and those who consumed food from the restaurant on the next day (33.1 ± 14.2 h) ($P = 0.32$).

Groups A and F had box lunches prepared by restaurant J and commercially available tea in a plastic bottle and consumed the box lunches on a ferry and a train, respectively. The same food items were assorted in the box lunches for these two groups. All other groups had lunch at restaurant J and had cold tea prepared by the restaurant. Although the combination of foods was not always identical, most foods were common in the lunches served to each group. When analysis was performed for each group separately, illness was statistically significantly associated with a specific food in three groups: Sara-Udon (thin fried rice noodles with mixed vegetables and seafood) in group C (OR, 3.1; 95% CI, 1.1 to 8.7; $P = 0.03$), deep-fried spring roll in group E1 (OR, 2.3; 95% CI, 1.1 to 4.7; $P = 0.02$), and boiled broccoli in group F (OR, 2.4; 95% CI, 1.2 to 4.6; $P = 0.01$). When analysis was performed for all subjects stratified together by group and day, deep-fried spring roll (Mantel-Haenszel OR, 2.06; 95% CI, 1.39 to 3.05; $P = 0.0004$), boiled broccoli (Mantel-Haenszel OR, 2.41; 95% CI, 1.29 to 4.51; $P = 0.009$), and raw lettuce (Mantel-Haenszel OR, 2.12; 95% CI, 1.13 to 3.95; $P = 0.03$) were significantly associated with illness. It may deserve to be mentioned that the four food items described above, the Sara-Udon, deep-fried spring roll, boiled broccoli, and raw lettuce, were handled with bare hands after cooking or washing. However, none of the groups were served all four of these items together. When deep-fried spring roll, boiled broccoli, and raw lettuce were included in the same model simultaneously, only boiled broccoli was significantly associated with illness (multivariate OR, 2.0; 95% CI, 1.0 to 3.9; $P = 0.05$) in groups A and F, to which all three of these

food items were served. However, none of these items that was significantly associated with illness was common to all groups.

There were two reports on the occurrence of secondary cases, besides the tourists: (i) NVs were detected in 2 sick employees of the hotel where group E stayed on the trip after visiting Nagasaki City, and (ii) 21 family members of 16 case patients in group C became sick.

Environmental investigation. On 14 November, the chief cook who was in charge of food hygiene at the kitchen had quit his job. This loss of staff, together with an extraordinary number of guests, made the business in the kitchen of the restaurant hectic during the 2-day period. One of the cooks felt general fatigue from 16 November and took an over-the-counter cold medicine on 19 November, although he allegedly had no gastrointestinal symptoms. No other restaurant staff allegedly had any illness during or immediately before the event. None of the employees reported that they had eaten raw shellfish, such as oysters, during the several days prior to the outbreak, and no family members of the employees were sick. All kitchen staff had eaten at least one meal at restaurant J on 18 and/or 19 November.

Restaurant J had only one washroom, which was located adjacent to the kitchen and which was used by both employees and tourists. Since there was no sink for hand-washing in the kitchen, the cooks washed their hands in the sink used to wash vegetables and kitchenware and wiped their hands on their aprons. The cooks and the other food handlers mostly handled the food items with their bare hands. Containers were commonly used for the food items before and after cooking. The same chopping board was used for different food items. The lettuce for the box lunches was washed with bare hands and soaked in water overnight, as was the boiled broccoli. The cold tea was prepared in a big bucket with hot water and then cooled with cubes of ice made in the ice machine in the kitchen.

In addition to the 29 environmental samples, a total of 58 meals served between 15 and 19 November were stored for the investigation and 9 bulk food items, such as frozen seafood, including bivalves similar to clams (*Paphia vernicosa*), had been kept during the inspection and were available for the investigation.

Microbiological investigation. Stool specimens (from 77 case patients) and vomit specimens (from 54 case patients) were obtained from a total of 124 case patients. Although *S. aureus* enterotoxins were detected in two vomit specimens from students in group E, the toxins from the two case patients were different: enterotoxin A and enterotoxin B, respectively. *Aeromonas hydrophila* was detected in a stool specimen from a case patient in group F. No enteropathogenic bacteria were detected in the other case patients, stool specimens from the kitchen staff, or the environmental samples from restaurant J.

RT-PCR and sequencing. Amplification by RT-PCR with genogroup-specific primers demonstrated the presence of 387-bp bands corresponding to GII NV (Fig. 2). GII NVs were detected in 87 of 124 case patients (70.2%; 44 of 54 vomit specimens [81.5%] and 48 of 77 stool specimens [62.3%]). No food samples were positive for NV, even after the nested PCR. Of the 29 environmental samples tested, only 1 was positive for GII NVs by the nested PCR (product size, 344 bp) (data not shown), and this sample was taken from the table where Sara-

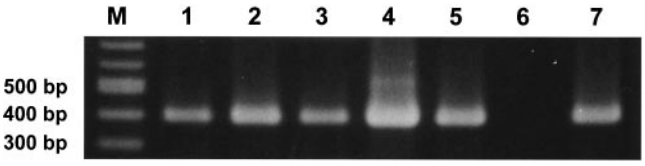

FIG. 2. Detection of NV capsid genes from specimens and samples by RT-PCR with genogroup-specific primers. The PCR products were electrophoresed on a 1.5% agarose gel. Lane M, marker (100-bp ladder; New England BioLabs Inc., Beverly, Mass.); lane 1, fecal specimen from the cook with general fatigue; lane 2, fecal specimen from another member of the kitchen staff (server); lanes 3 to 5, fecal specimens from representative case patients; lane 6, negative control (free of viral DNA); lane 7, positive control for genogroup II (strain Arg320; GenBank accession number AF190817). The GII NVs capsid gene (387 bp) was amplified and detected in the fecal specimens (lanes 1 to 5).

Udon was dished up. GII NVs were also detected in the stool specimens from 5 of 10 kitchen staff, including 2 cooks and 3 servers.

Real-time PCR quantification of the NVs revealed 61.5 copies/cm^2 in the table sample and 3.7×10^8 to 9.4×10^9 copies/g in the stool specimens from the kitchen staff. The capsid sequence analysis revealed that the NVs in all samples from the case patients, the kitchen staff, and the environmental sample had identical sequences (GenBank accession number AY590117). The genotype is most closely related to the well-characterized genotype Mexico/89/MX (GenBank accession number U22498), with 94.9% identity at the nucleotide sequence level (Fig. 3). The sequence in GenBank most closely related to the sequence that we obtained was Oberhausen455/01/DE (GenBank accession number AF425768), with which our sequence had 98.9% identity at the nucleotide level and which was originally from an outbreak in Germany.

DISCUSSION

To our knowledge, this is the largest food-borne gastroenteritis outbreak in terms of the distribution from a single causative facility into diverse geographic locations across the country, and the existence of an outbreak was unambiguously shown by linking classical and molecular epidemiological measures to a single GII NV strain of the Mexico genotype. Although recent papers have shown that new GII/4 NVs emerged in Europe (18) and on cruise ships in the Unites States (32), the causative NV in our study was classified as a different subtype, subtype GII/3. The outbreak described here is thought to be unique in that several tourist groups from across Japan were affected with gastroenteritis by exposure to NVs from a specific restaurant during a defined period of time and became ill at home or on the continuation of their trips; consequently, the specific virus has since spread into multiple prefectures. Such spread of a single infectious agent by travelers who play the role of disease transmission vehicle should be cautionary, as the outbreak is further proof of one of the contemporary modes of transmission of infectious diseases. Actually, Beller et al. (2) reported on a waterborne outbreak of illness caused by NVs in tourists traveling by bus between the United States and Canada. Furthermore, Noel et al. (25) reported that NV outbreaks due to a single virus occurred in seven countries on five continents during the 1995-1996 sea-

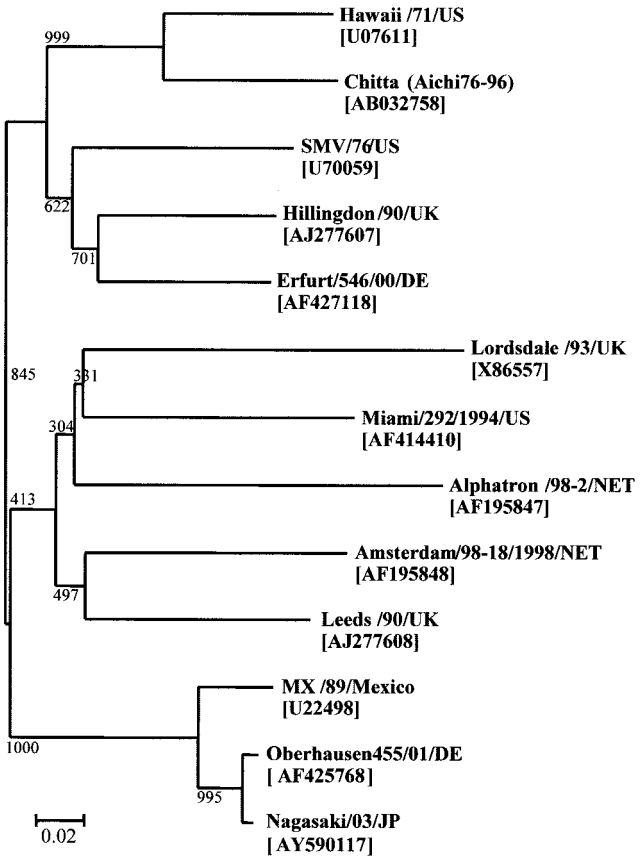

FIG. 3. Phylogenetic tree constructed on the basis of the sequences of a part of the capsid gene of GII NVs from the present outbreak and known strains from the GenBank database. GenBank accession numbers for the strains are indicated in the parenthesis. The causative viral strain of the present outbreak is shown as Nagasaki/03/JP. The numbers at each branch indicate bootstrap values for the clusters supported by that branch.

son, suggesting that the circulation of the strains might involve patterns of transmission not previously considered.

Food-borne vehicles of NVs are typically contaminated bivalve shellfish, such as oysters, items contaminated by infected food handlers, or vegetables or fruit contaminated by irrigation or washing (20). In restaurant J, frozen imported bivalve shellfish was initially suspected as the cause of infection, but no NV was detected in either the shellfish or other food samples. In outbreaks originating from infected food handlers, specific food is not always identified as the main source of the infection (5, 12, 15). Lopman et al. (19) have recently reported that specific vehicles were implicated in 39.1% of NV food-borne outbreaks and that multiple food vehicles contributed to some outbreaks. In the present outbreak, it is still unknown whether a sick cook was first infected with NV and subsequently other kitchen staff and tourists were infected or whether the kitchen staff was infected simultaneously with tourists by unknown transmission routes. However, we believe that several foods were contaminated by employees working at restaurant J. This is supported by the facts that (i) identical NVs were detected from the kitchen staff, the kitchen environment, and case patients; (ii) no NVs were found in meal or food samples; (iii) no common foods were suspected as the main source of infection; (iv) there were no differences in the attack rates between groups of tourists who ate box lunches prepared by the restaurant and those who ate at the restaurant, even though there was a great difference in the combinations of foods consumed; (v) the kitchen staff mostly handled food items with bare hands; and (vi) the kitchen staff used poor food-handling hygiene.

Although we failed to obtain a sample of ice tea prepared by the restaurant, the attack rate among the students who consumed commercially available bottled tea did not differ at all from that among those who consumed ice tea prepared by the restaurant, suggesting that waterborne transmission was much less likely. Indirect contamination in the washroom was also less likely because NV was not detected in the washroom and the illness occurred in the tourists who did not visit the restaurant. Unfortunately, we failed to obtain a sample of water in which the lettuce and broccoli were soaked overnight. The attack rates were significantly lower in the tourists who ate food from the restaurant on 19 November than those who ate food from the restaurant on 18 November ($P < 0.001$), and the rates dropped steeply on 19 November, suggesting that the foods were substantially more contaminated on 18 November (Tables 1 and 2). The fact that the attack rates for groups E1 and E2 (77.6 and 21.4%, respectively), which had the same background, showed a significant difference ($P < 0.001$) supports this hypothesis (Tables 1 and 2).

Although the highest incidence of NV infections is in children under 5 years, NV infections can occur at any age (20). In the outbreak reported here, all tourists ate a similar combination of foods at the restaurant, while the attack rates for adult tourist groups were much lower than those for student tourist groups. This suggests that NV gastroenteritis may tend to cause more severe illness in children and adolescents than in adults. This is consistent with the findings of a proportion analysis study conducted in The Netherlands (4), which showed that individuals in the age group of 18 to 64 years demonstrated a lower infection rate than individuals in younger and older age groups. Although the average incubation time in the present outbreak was thought to be typical for primary NV gastroenteritis, it is possible that some cases with apparently longer incubation periods were probably due to secondary person-to-person transmission, since most tourist groups continued their tours after they left Nagasaki City.

The sudden emergence and spread of a single strain raise important public health implications about the mode of transmission that permitted the rapid radiation of a single virus (6). It is generally believed that the movement of people from one place to another, whether it is through tourism or other means, may have profound effects on the dissemination of NVs into different populations, but there is not much evidence that directly supports such a hypothesis. In this regard, this study provides a unique opportunity to gain insight into the question of how various genotypes of NVs emerge, cocirculate, and disappear in different geographic locations. It is important and interesting to use modern molecular biology-based techniques to keep track of where this NV outbreak strain will spread and if it will cause outbreaks in Japan or elsewhere in the world. For this purpose, enhanced vigilance that includes the pursuit and characterization of secondary cases that follow outbreak

cases is continuously needed. It is also essential that food samplers not work when they are ill and that good handwashing facilities be provided in all restaurants.

ACKNOWLEDGMENTS

We thank the epidemiologists and laboratory staff at the local public health centers and the Institute of Public Health and Environmental Sciences for collaboration on the investigation of this outbreak. We thank Masahiro Eguchi and Yoko Yamaguchi, Food Hygiene Section of the Nagasaki City Health Department, for helpful assistance in collecting epidemiologic information and data preparation.

The authors do not have any commercial or other association that might pose a conflict of interest.

The authors are members of the Committee of Investigation into Cause of Food-Poisoning at Restaurant J, Nagasaki City (chaired by Osamu Nakagomi); and the remaining members are Kohji Hirano (Nagasaki Prefectural Social Welfare and Human Services Department), Takeshi Hara (Nagasaki Prefectural Institute of Public Health and Environmental Sciences), and Shizumune Matsuda (Nagasaki City Public Health Center).

REFERENCES

1. **Ando, T., J. S. Noel, and R. L. Fankhauser.** 2000. Genetic classification of "Norwalk-like viruses." J. Infect. Dis. **181**(Suppl. 2)**:**S336–S348.
2. **Beller, M., A. Ellis, S. H. Lee, M. A. Drebot, S. A. Jenkerson, E. Funk, M. D. Sobsey, O. D. Simmons III, S. S. Monroe, T. Ando, J. Noel, M. Petric, J. P. Middaugh, and J. S. Spika.** 1997. Outbreak of viral gastroenteritis due to a contaminated well. International consequences. JAMA **278:**563–568.
3. **Dedman, D., H. Laurichesse, E. O. Caul, and P. G. Wall.** 1998. Surveillance of small round structured virus (SRSV) infection in England and Wales, 1990-5. Epidemiol. Infect. **121:**139–149.
4. **de Wit, M. A., M. P. Koopmans, L. M. Kortbeek, W. J. Wannet, J. Vinje, F. van Leusden, A. I. Bartelds, and Y. T. van Duynhoven.** 2001. Sensor, a population-based cohort study on gastroenteritis in The Netherlands: incidence and etiology. Am. J. Epidemiol. **154:**666–674.
5. **Dippold, L., R. Lee, C. Selman, S. Monroe, and C. Henry.** 2003. A gastroenteritis outbreak due to norovirus associated with a Colorado hotel. J. Environ. Health **66:**13–17, 26.
6. **Fankhauser, R. L., J. S. Noel, S. S. Monroe, T. Ando, and R. I. Glass.** 1998. Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastroenteritis in the United States. J. Infect. Dis. **178:**1571–1578.
7. **Froggatt, P. C., I. B. Vipond, C. R. Ashley, P. R. Lambden, I. N. Clarke, and E. O. Caul.** 2004. Surveillance of norovirus infection in a study of sporadic childhood gastroenteritis in South West England and South Wales, during one winter season (1999-2000). J. Med. Virol. **72:**307–311.
8. **Glass, R. I., J. Noel, T. Ando, R. Fankhauser, G. Belliot, A. Mounts, U. D. Parashar, J. S. Bresee, and S. S. Monroe.** 2000. The epidemiology of enteric caliciviruses from humans: a reassessment using new diagnostics. J. Infect. Dis. **181**(Suppl. 2)**:**S254–S261.
9. **Hohne, M., and E. Schreier.** 2004. Detection and characterization of norovirus outbreaks in Germany: application of a one-tube RT-PCR using a fluorogenic real-time detection system. J. Med. Virol. **72:**312–319.
10. **Inouye, S., K. Yamashita, S. Yamadera, M. Yoshikawa, N. Kato, and N. Okabe.** 2000. Surveillance of viral gastroenteritis in Japan: pediatric cases and outbreak incidents. J. Infect. Dis. **181**(Suppl. 2)**:**S270–S274.
11. **Iritani, N., Y. Seto, H. Kubo, T. Murakami, K. Haruki, M. Ayata, and H. Ogura.** 2003. Prevalence of Norwalk-like virus infections in cases of viral gastroenteritis among children in Osaka City, Japan. J. Clin. Microbiol. **41:**1756–1759.
12. **Johansson, P. J., M. Torven, A. C. Hammarlund, U. Bjorne, K. O. Hedlund, and L. Svensson.** 2002. Food-borne outbreak of gastroenteritis associated with genogroup I calicivirus. J. Clin. Microbiol. **40:**794–798.
13. **Kageyama, T., S. Kojima, M. Shinohara, K. Uchida, S. Fukushi, F. B. Hoshino, N. Takeda, and K. Katayama.** 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J. Clin. Microbiol. **41:**1548–1557.
14. **Katayama, K., H. Shirato-Horikoshi, S. Kojima, T. Kageyama, T. Oka, F. Hoshino, S. Fukushi, M. Shinohara, K. Uchida, Y. Suzuki, T. Gojobori, and N. Takeda.** 2002. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. Virology **299:**225–239.
15. **Kobayashi, S., T. Morishita, T. Yamashita, K. Sakae, O. Nishio, T. Miyake, Y. Ishihara, and S. Isomura.** 1991. A large outbreak of gastroenteritis associated with a small round structured virus among schoolchildren and teachers in Japan. Epidemiol. Infect. **107:**81–86.
16. **Kojima, S., T. Kageyama, S. Fukushi, F. B. Hoshino, M. Shinohara, K. Uchida, K. Natori, N. Takeda, and K. Katayama.** 2002. Genogroup-specific PCR primers for detection of Norwalk-like viruses. J. Virol. Methods **100:** 107–114.
17. **Koopmans, M., J. Vinje, M. de Wit, I. Leenen, W. van der Poel, and Y. van Duynhoven.** 2000. Molecular epidemiology of human enteric caliciviruses in The Netherlands. J. Infect. Dis. **181**(Suppl. 2)**:**S262–S269.
18. **Lopman, B., H. Vennema, E. Kohli, P. Pothier, A. Sanchez, A. Negredo, J. Buesa, E. Schreier, M. Reacher, D. Brown, J. Gray, M. Iturriza, C. Gallimore, B. Bottiger, K. O. Hedlund, M. Torven, C. H. von Bonsdorff, L. Maunula, M. Poljsak-Prijatelj, J. Zimsek, G. Reuter, G. Szucs, B. Melegh, L. Svennson, Y. van Duijnhoven, and M. Koopmans.** 2004. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet **363:**682–688.
19. **Lopman, B. A., G. K. Adak, M. H. Reacher, and D. W. Brown.** 2003. Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992-2000. Emerg. Infect. Dis. **9:**71–77.
20. **Lopman, B. A., D. W. Brown, and M. Koopmans.** 2002. Human caliciviruses in Europe. J. Clin. Virol. **24:**137–160.
21. **Lopman, B. A., M. H. Reacher, Y. Van Duijnhoven, F. X. Hanon, D. Brown, and M. Koopmans.** 2003. Viral gastroenteritis outbreaks in Europe, 1995-2000. Emerg. Infect. Dis. **9:**90–96.
22. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
23. **Nicollier-Jamot, B., V. Pico, P. Pothier, E. Kohli, B. K. Bohnker, and S. Thornton.** 2003. Molecular cloning, expression, self-assembly, antigenicity, and seroepidemiology of a genogroup II norovirus isolated in France. J. Clin. Microbiol. **41:**3901–3904.
24. **Nishida, T., H. Kimura, M. Saitoh, M. Shinohara, M. Kato, S. Fukuda, T. Munemura, T. Mikami, A. Kawamoto, M. Akiyama, Y. Kato, K. Nishi, K. Kozawa, and O. Nishio.** 2003. Detection, quantitation, and phylogenetic analysis of noroviruses in Japanese oysters. Appl. Environ. Microbiol. **69:** 5782–5786.
25. **Noel, J. S., R. L. Fankhauser, T. Ando, S. S. Monroe, and R. I. Glass.** 1999. Identification of a distinct common strain of 'Norwalk-like viruses' having a global distribution. J. Infect. Dis. **179:**1334–1344.
26. **Otsu, R., A. Ishikawa, K. Mukae, H. Nakayama, and M. Sarashi.** 2003. Molecular epidemiology of Norwalk-like virus (NLV) outbreaks occurring in Kyushu Japan between 1988 and 1993. Eur. J. Epidemiol. **18:**369–372.
27. **Pang, X. L., S. Honma, S. Nakata, and T. Vesikari.** 2000. Human caliciviruses in acute gastroenteritis of young children in the community. J. Infect. Dis. **181**(Suppl. 2)**:**S288–S294.
28. **Tompkins, D. S., M. J. Hudson, H. R. Smith, R. P. Eglin, J. G. Wheeler, M. M. Brett, R. J. Owen, J. S. Brazier, P. Cumberland, V. King, and P. E. Cook.** 1999. A study of infectious intestinal disease in England: microbiological findings in cases and controls. Commun. Dis. Public Health **2:**108–113.
29. **Vinje, J., J. Green, D. C. Lewis, C. I. Gallimore, D. W. Brown, and M. P. Koopmans.** 2000. Genetic polymorphism across regions of the three open reading frames of "Norwalk-like viruses." Arch. Virol. **145:**223–241.
30. **Vinje, J., H. Vennema, L. Maunula, C. H. von Bonsdorff, M. Hoehne, E. Schreier, A. Richards, J. Green, D. Brown, S. S. Beard, S. S. Monroe, E. de Bruin, L. Svensson, and M. P. Koopmans.** 2003. International collaborative study to compare reverse transcriptase PCR assays for detection and genotyping of noroviruses. J. Clin. Microbiol. **41:**1423–1433.
31. **Wang, J., X. Jiang, H. P. Madore, J. Gray, U. Desselberger, T. Ando, Y. Seto, I. Oishi, J. F. Lew, K. Y. Green, et al.** 1994. Sequence diversity of small, round-structured viruses in the Norwalk virus group. J. Virol. **68:**5982–5990.
32. **Widdowson, M. A., E. H. Cramer, L. Hadley, J. S. Bresee, R. S. Beard, S. N. Bulens, M. Charles, W. Chege, E. Isakbaeva, J. G. Wright, E. Mintz, D. Forney, J. Massey, R. I. Glass, and S. S. Monroe.** 2004. Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus—United States, 2002. J. Infect. Dis. **190:**27–36.
33. **Yan, H., F. Yagyu, S. Okitsu, O. Nishio, and H. Ushijima.** 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.