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Food-Borne Outbreak of Gastroenteritis Associated with Genogroup I Calicivirus

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An outbreak of gastroenteritis affecting 158 of 219 (72%) guests and employees at a hotel is described. Food served at the hotel restaurant is believed to have been the source of the outbreak and to have been contaminated by sick employees working in the restaurant. A secondary attack rate of 22% was seen involving 43 persons in all. In stool specimens from seven of eight patients, Norwalk-like viruses (NLVs) were detected by electron microscopy. While NLV-specific PCR using primers JV12 and JV13 were negative, all specimens examined with primers NVp69 and NVp110 were positive. The failure of primers JV12 and JV13 was attributed to several mismatches in the JV12 primer. Genotyping and sequence analysis revealed that all samples had identical sequences and clustered with genogroup I, and the most closely related well-characterized genotype is Desert Shield. This is the first described food-borne outbreak associated with genogroup I virus in Sweden.

Since the discovery of the Norwalk virus in 1972 (26), Norwalk-like viruses (NLVs) have been recognized as the leading cause of acute nonbacterial gastroenteritis (6, 10, 15, 27, 36). The Norwalk virus was cloned and sequenced in the early 1990s and was found to belong to the human Caliciviridae (20, 23). On the basis of their sequences within the putative RNA-dependent RNA polymerase region and the capsid region, members of the genetically and antigenetically diverse NLVs can be divided into the two genogroups I and II (2, 13, 39). The genogroups can be further divided into various genotypes, but whether the various genogroups and genotypes differ in epidemiological, clinical, or other characteristics is presently unknown.

NLV-associated gastroenteritis is transmitted by the fecoral route and occurs both as sporadic community cases and as large outbreaks in, e.g., nursing homes, hospitals, schools, universities, vacation camps, cruise ships, hotels, and restaurants (10, 27). Many of the sporadic cases in the community are spread by person-to-person contacts, while outbreaks often are associated with the ingestion of food or water contaminated by the virus (4, 7, 12, 14, 25, 28).

Here we describe a food-borne outbreak of gastroenteritis associated with a genogroup I, Desert Shield-like calicivirus. Desert Shield virus was first isolated from U.S. military troops who had gastroenteritis while stationed in Saudi Arabia during operation Desert Shield in 1990 (17), and it was characterized a few years later (30). Transmission of the Desert Shield-like calicivirus has been suggested to occur by contaminated water as well as by person-to-person spread (11, 18, 33). We believed that our outbreak originated from an infected food handler.

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trontestinal symptoms was defined as a patient with one or more of the following symptoms: nausea, vomiting, abdominal cramping, and/or diarrhea. A patient with gastroenteritis was defined as a patient with vomiting and/or diarrhea.

Environmental investigation. Public health inspectors conducted an environmental assessment of the hotel restaurant and collected samples of foods served on 2 and 3 May (ground beef, chicken, vegetarian paella, goulash, salads, and desserts). The food samples were analyzed for pathogenic food-borne bacteria like Salmonella, Escherichia coli, Enterobacteraeaceae, Staphylococcus, Bacillus cereus, and Clostridium perfringens and for yeast and mold species.

Laboratory investigation. Bacterial cultures of stool samples from eight patients (seven hotel guests and one employee) were performed at the clinical microbiological laboratory at the University Hospital in Lund, Sweden. The stool samples were cultured for Salmonella, Shigella, Campylobacter, and Yersinia species.

EM. Electron microscopy (EM) was performed essentially as previously described (15). Briefly, 10% fecal suspension was prepared in phosphate-buffered saline, and a drop of the suspension was incubated for 1 min on a Formvar-carbon-coated grid and then stained with 2% tungstophosphoric acid. To increase the sensitivity, a second step was performed in which the fecal suspension was clarified at 20,000 × g for 30 min, and the supernatant was pelleted directly on the grid at 150,000 × g for 10 min in a Beckman (Palo Alto, Calif.) Airfuge.

RNA extraction. RNA was extracted from 100 μl of a 10% fecal suspension by use of the guanidinium thiocyanate-silica extraction method (5).

Detection of NLVs by RT-PCR. Reverse transcriptase (RT)-PCR was performed with the primer pair JV12-JV13, which generates a 326-bp product (36, 37), and with primers designated NVp69 (upstream; 5′-GGC CTG CCA TCT GGA TGG CC-3′) and NVp110 (downstream; 5′-AC[A/T/G] AT[C/T] TCA TCA TCA CCA TA-3′), which yield a 151-bp fragment of the RNA polymerase region (nucleotides [nt] 4753 to 4884, corresponding to equivalent locations within the Norwalk sequence, M87661) (29).

Genotyping. All PCR-positive samples were genotyped by reverse line blot hybridization essentially as described previously (38) with the modification that primer pair NVp110-NVp69, with primer NVp110 biotinylated, was used instead of primer pair JV12-JV13.

Sequencing. The RT-PCR-positive samples were further characterized by sequence analysis using the NVp69 primer and the ABI Prism BigDye Terminator Cycle-Sequencing Ready Reaction kit on an ABI 310 automated sequencer. Sequences were edited using Seq Ed version 1.0.3 software and aligned using Se-al software. A 108-nt stretch of the RNA polymerase region was obtained and aligned against sequences obtained from the GenBank database. The dendrogram was produced by PAUP version 3.1.1 software.

In order to investigate why primer pair JV12-JV13 failed to amplify the samples, a new primer was designed to cover the JV12 region. The BLAST search program was used to find the sequence in GenBank most closely related to the sequence obtained by NVp69. A new primer (5′-TGC TAT AGA GGA TGG TGG CCC TT-3′) was designed from this sequence and used in RT-PCR together with primer JV13, resulting in a 375-bp fragment. This fragment was sequenced with the JV13 primer, as described above, and a 329-nt stretch of the polymerase region was obtained.

Statistical analysis. For statistical analysis, the nonparametric chi-square test and Fisher’s exact test were used.

Nucleotide sequence accession number. Sequence data have been submitted to GenBank and assigned accession number AF356599.

RESULTS

Incidence and clinical symptoms. Information was obtained from 219 of the 246 (89%) contacted guests and employees; 214 responded to the questionnaire, and for an additional 5, information was obtained by interview. In all, 158 of the 219 (72%) guests and employees fell ill with clinical symptoms like nausea (89%), vomiting (77%), diarrhea (74%), abdominal cramping (61%), headache (39%), fever (30%), and myalgias (27%) (Table 1). There were no significant differences in attack rate, symptoms, or onset and duration of disease for the 78 guests living at the hotel and the 80 guests, of which 46 were hospital employees, only eating at the restaurant. A total of 156 patients had gastrointestinal symptoms (nausea, vomiting, gastrointestinal cramping, and/or diarrhea), and 147 had gastroenteritis (vomiting and/or diarrhea).

Patients with gastroenteritis. The first to fall ill with gastroenteritis was the chef of the restaurant on 2 May, followed the next day by five employees, all working in the restaurant kitchen. In all, 9 of 11 employees working in the kitchen had gastroenteritis on that and the following days. On 3 May, guests who had eaten at the restaurant, but with no direct contact with the kitchen, started to fall ill. The dates of onset of gastroenteritis for guests and employees were from 2 to 10 May, with a peak on 4 May (Fig. 1). Of the 147 patients with gastroenteritis, 98 (67%) were females and 49 (33%) were males. The mean and median ages were 46 and 45 years, respectively, with a range of 1 to 83 years. The age distribution can be seen in Fig. 1; one patient was below 10 years of age (14 months old), and two patients were >80 years old (82 and 83 years old).

The duration of symptoms was known for 139 of the 147 patients. It was less than 24 h for 37 of the patients, 24 to 48 h for 61 patients, 48 to 72 h for 27 patients, and more than 72 h for 14 patients. One patient had to receive intravenous fluids to treat dehydration, but all patients recovered.

The food served at the hotel restaurant was suspected to be contaminated and the source of the outbreak. For 211 of the 219 guests and employees, information about whether they had been eating at the restaurant on 2 and/or 3 May was available. In all, 200 of the 211 had been eating at the restaurant, and of these, 142 (71%) had fallen ill with gastroenteritis while 58 (29%) remained well. A significant correlation was seen be-

| TABLE 1. Clinical symptoms of patients falling ill from a food-borne calicivirus infection |
|-----------------------------------------------|-----|
| Symptom                                         | No. of patients (percentage) |
| Clinical symptoms                                | 158 |
| Nausea                                          | 140 (89) |
| Vomiting                                        | 121 (77) |
| Diarrhea                                        | 117 (74) |
| Abdominal cramping                              | 96 (61) |
| Headache                                        | 61 (39) |
| Fever                                           | 48 (30) |
| Myalgia                                         | 43 (27) |
| Gastrointestinal symptoms*                      | 156 |
| Gastroenteritis*                                 | 147 |

* Defined as nausea, vomiting, diarrhea and/or gastrointestinal pains.
* Defined as vomiting and/or diarrhea.

FIG. 1. Onset of symptoms for 147 patients with gastroenteritis from 2 to 10 May 2000.
between eating at the restaurant and falling ill with gastroenteritis ($P < 0.001$; Fisher’s exact test), but no specific food could be associated with disease. Of the 58 guests who remained well despite eating at the restaurant, some had avoided the salads while others had eaten the same food as those who had fallen ill. Only one patient with gastroenteritis had not been eating at the restaurant. This patient worked as a cleaning person at the hotel.

For 61 of the patients with gastroenteritis who had been eating at the restaurant on either 2 or 3 May, an incubation period could be calculated. All of these patients fell ill within 2 days after eating at the restaurant; 2 patients fell ill on the day they ate at the restaurant, 44 patients fell ill the day after, and 15 patients fell ill 2 days after eating at the restaurant. This suggests that the infection was food borne until 6 May (Fig. 1) and thereafter was transmitted by person-to-person contact or from a contaminated environment.

Secondary gastrointestinal infections. Of the 147 patients with gastroenteritis, 117 had social contacts with one or more persons, mostly family members, with no direct connection to the restaurant or hotel; 26 of these contacts resulted in secondary gastrointestinal infection (a secondary attack rate of 22%) affecting 43 persons in all. All secondarily infected persons were identified on clinical grounds alone, and no genotype identification was performed. The mean age of the secondarily infected persons was 36 years, with a range of 2 to 80 years. No case of gastroenteritis was observed among the 13 newborns at the hotel, although five parents had symptoms.

One probable secondary case was seen among patients at the university hospital. Two of the hotel guests with gastroenteritis had to be transferred to the cardiology department at the hospital, and one of them shared a wardroom with another patient who 2 days later had vomiting and diarrhea. Although no diagnostic tests were performed, this was judged to be a probable secondary case of calicivirus infection.

Environmental investigation. No pathogenic bacteria, such as *Salmonella*, *Shigella*, *Campylobacter*, or *Yersinia* species. In stool specimens from seven of the eight patients, NLVs were identified by EM. Six of the seven positive specimens contained sufficient material to also be examined by an NLV-specific PCR utilizing primers JV12 and JV13 (37), and surprisingly, these primers failed to give a positive reaction. This led us to retest with a second set of NLV-specific primers designated NVp69 and NVp110, which gave a specific reaction in six of six specimens. Sequence analysis revealed that primer JV12 had six mismatches with the target sequence and primer JV13 had two mismatches, which may explain the failure with these primers.

A modified reverse line blot hybridization assay (38) including primer pair NVp110-NVp69, with primer NVp110 biotinylated, was then used to genotype the six specimens as Desert Shield-like viruses. Sequence analysis of the six RT-PCR-positive samples showed that all of the samples had identical sequences (321 nt) and clustered with genogroup I and the most closely related Desert Shield virus with 82% identity on the nucleotide level (Fig. 3). The BLAST search showed that the strain in GenBank most closely related to the sequence obtained by NVp69 and JV13 was from a food-borne outbreak in Hokkaido, Japan, where oysters were the probable source of infection (accession number ABO19262). It has 98% identity on the nucleotide level.

**DISCUSSION**

NLVs represent the major cause of acute nonbacterial gastroenteritis outbreaks among adults and the elderly (6, 10, 15, 796 JOHANSSON ET AL. J. CLIN. MICROBIOL.)
we made this observation we have identified
routine diagnostic work in Sweden, where most strains, how-
sequence. The fact that the primer pair designated JV12-JV13
sequencing of samples from six patients revealed the same
that caliciviruses were detected by EM and PCR and that
guests and employees. The diagnosis was based on the facts
hospital in southern Sweden and involving 158 of 219 (72%)
has up to now been the dominant genotype (unpublished).
22, 32). In Sweden, genogroup II, Lordsdale-like viruses (8),
types as more extensive information becomes available (1, 3,
lecular methods has made it possible to characterize different
viruses in patients with gastroenteritis has been the examina-
72 h, but for some the duration of the symptoms was rather
prominent symptoms in most affected individuals, but other
eral in this outbreak were consistent with those reported in
restaurant who had eaten the same food either fell ill or re-
ill within 2 days after eating at the restaurant, which is in
agreement with the known incubation period for caliciviruses
(27). This makes it probable that the infection after 5 May was
no longer food borne but was transmitted either by person-to-
person contact or from the environment.
We were not able to identify any specific food served at the
restaurant as the main source of the infection. Guests in the
restaurant who had eaten the same food either fell ill or re-
ained well. The differences in outcome could be due to vari-
ations in infection dose or in immunity to this specific variant
of calicivirus. Antibodies to NLVs are acquired in early child-
hood and can be detected in up to 90% of older children and
adults (16, 35). Studies of the role of serum antibodies in
mediating protection against NLVs, however, have yielded
conflicting results. In adults, preexisting serum antibodies do
not seem to be associated with protective immunity, but anti-
body levels become associated with protection after repetitive
exposure (24).
Very few viruses are needed for infection, and therefore,
transmission is possible by droplets and person-to-person con-
tacts and from a contaminated environment, as well as from
contaminated food. The only patient with gastroenteritis who
did not eat at the restaurant worked as a cleaning person at the
hotel and could have been infected from the environment when
cleaning up after sick guests. It is known that caliciviruses can be
persistent in the environment for a rather extended time and
that they are relatively resistant to a variety of disinfectants (9).
Secondary transmission and a high secondary attack rate for
family members and friends is a prominent feature of calicivi-
ruses (27). We noted a secondary attack rate of 22% affecting
mostly family members. Only one secondary case was seen at
the university hospital, which may be attributed to the fact that
guests at the hotel, as soon as an outbreak was suspected, were
prohibited from visiting the hospital. Additionally, medical
staff who had eaten at the restaurant and who worked at the
hospital were sent home immediately at the slightest sign of
gastrointestinal symptoms.
This is the first recorded outbreak of a Desert Shield-like
virus in Sweden. Our data illustrate the limitations of using
only one pair of PCR primers for diagnostic purposes and
point to the importance of good food hygiene practices and the
immediate exclusion of infected food handlers from work.

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