

CONCISE COMMUNICATION

A Foodborne Outbreak of Gastroenteritis Associated with Norwalk-like Viruses: First Molecular Traceback to Deli Sandwiches Contaminated during Preparation

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In March 1998, an outbreak of acute gastroenteritis occurred among students at a Texas university. Overall, 125 ill students sought medical care. Case-control studies revealed that illness was significantly associated with eating foods from the university's main cafeteria deli bar on 9 and 10 March. Stool specimens from 9 (50%) of 18 ill students and samples of deli ham showed evidence of Norwalk-like viruses (NLVs) by reverse-transcriptase (RT) polymerase chain reaction (PCR) assay. A food handler who prepared sandwiches for lunch on 9 March reported that her infant had been sick with watery diarrhea since just before the outbreak. A stool sample from the infant was positive for NLV by RT-PCR, and the sequence of the amplified product was identical to that of amplified product from deli ham and students' stool specimens. This is the first time RT-PCR and sequence analysis have successfully confirmed viral contamination of a food item likely to have been contaminated by a food handler.

Norwalk-like viruses (NLVs) are a leading cause of acute gastroenteritis worldwide [1]. Since the identification of Norwalk virus in 1972, numerous outbreaks of gastroenteritis from NLVs have been recognized. NLVs can be transmitted through contaminated food or water, directly from person to person, and occasionally by airborne droplets produced during vomiting [2]. Food handlers are often suspected as the source of foodborne outbreaks, and there have been reports of possible NLV transmission during both pre- [3] and postsymptomatic periods [4].

Genomic characterization of viruses within the family Caliciviridae has allowed the development of reverse-transcriptase (RT) polymerase chain reaction (PCR) assays that are highly sensitive and widely used in the testing of clinical stool specimens for NLVs [5–7]. The utility of RT-PCR for direct detection of NLV in contaminated foods other than seafood has only recently been described [8]. Although NLVs can be identified by immune electron microscopy, the lack of a more specific assay to detect viral contamination of foods has often limited epidemiologic investigations during outbreaks.

On 10 and 11 March 1998, emergency room personnel at a Texas hospital treated 23 students from a local university for acute gastroenteritis. We investigated the outbreak to identify the cause and source of illness, making use of an RT-PCR assay and sequencing to detect calicivirus in implicated food items and in stool samples from patients and the child of a food handler.

Methods

Epidemiologic investigations. After hypothesis-generating interviews with selected ill students, we conducted matched and unmatched case-control studies. In both studies, a case was defined

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as acute gastroenteritis in a student who sought care at either an emergency room or the student health service during 5–13 March 1998. In the first case-control study (the matched study), controls were well dormitory roommates of case-patients. A questionnaire was used to obtain information on meals and specific foods eaten during 4–10 March. The second, unmatched case-control study was conducted to further assess risks associated with vehicles identified during the first case-control study, again with use of a written questionnaire. Controls were randomly selected from a dining hall database that indicated their attendance at one of several meals implicated in the matched case-control study. Students interviewed during the first case-control study were excluded from the second case-control study. Data were analyzed by use of Epi-Info version 6.04a software (Centers for Disease Control and Prevention [CDC], Atlanta).

Environmental investigation. State and local inspectors conducted an environmental assessment of the university's main cafeteria and collected samples of leftover food. All dining service personnel were interviewed about recent gastrointestinal illness before and during the outbreak and were asked about their work responsibilities.

Laboratory investigation. Blood chemistry testing and bacterial cultures were performed at a local hospital, and stool bacterial results were confirmed at the Texas Department of Health laboratory. Virus studies done at the CDC included RT-PCR for NLVs with use of the G1 and G2 primer sets in the polymerase region [6] and primers mon381 and mon383 in the capsid region [9]. A subset of the positive samples was characterized by sequence analysis.

Food samples (ham sliced on both 9 and 10 March, turkey and salami sliced on-site on 10 March, and lettuce salad made on-site on 12 March) collected from the deli bar were sent to the Division of Molecular Virology at Baylor College of Medicine (Houston), refrigerated, and assayed for the presence of NLV. Food samples were processed for recovery of virus by means of a newly developed guanidinium-based method [8]. In brief, ~30 g of each food sample was placed in a sterile container and rinsed twice with 4 mL of TRIzol (Life Technologies Gibco BRL, Gaithersburg, MD). The wash retentate was collected and clarified by centrifugation, and the upper lipid layer and residual food pellet were discarded. The aqueous layer was extracted after the addition of chloroform, and the viral RNA-containing aqueous phase was then precipitated in isopropanol. The resulting pellet was washed with 70% ethanol, air dried, and suspended in 100 μ L of RNase-free water. Twenty-microliter samples of 10- and 100-fold dilutions of recovered RNA were analyzed by RT-PCR with use of NLV-specific primers targeting the polymerase [10] and capsid regions [9]. PCR products were detected by Southern hybridization [11] with NLV-specific probes that produced positive results in stool samples from this outbreak. A previously described internal standard was used as a positive RNA control to determine whether negative results might be due to the presence of inhibitors in the samples subjected to RT-PCR amplification [11]. The RT-PCR hybridization-positive food sample was further characterized by nested PCR amplification of the capsid region, followed by direct sequencing of the resulting amplicons to determine relatedness to the clinical specimens. To minimize the risk of carryover contamination, nested PCR amplification using entirely different sets of reagents and equipment (en-

zymes, buffers, tubes, pipettors, and thermal cycler) was done in a different wing of the building.

Results

Epidemiologic investigations. All identified cases occurred among students who lived on campus and ate most of their meals at the university's main cafeteria. Of the 2054 university students participating in the university meal plan, 61 presented to the student health service with symptoms of acute gastroenteritis, and 64 others presented to a local emergency room. Of the 125 students who sought treatment, 65 (52%) were interviewed for the case-control studies. The dates of illness onset for the students were 6–14 March 1998, with a peak on 10 March (figure 1).

Symptoms of ill persons included vomiting (91%), diarrhea (85%), abdominal cramping (68%), headache (66%), myalgias (49%), and bloody diarrhea (5%). The oral temperatures recorded for students in the emergency room ranged from 37.1°C to 39.1°C. The median white blood cell count was 13.7/mm³ (range, 5.4–22.7), with 82% polymorphonuclear cells, 6% lymphocytes, and 7% band forms. Fecal leukocytes were identified in 11 (58%) of 19 stool specimens tested, and fecal occult blood was identified in 5 (26%). The median duration of illness was 2 days (range, 1–10), and the median number of class days missed was 2 (range, 0–3). All 23 students hospitalized for severe dehydration recovered.

In the matched case-control study, illness was associated with eating at the main cafeteria deli bar during lunch on 9 March (odds ratio [OR], 11.0; 95% confidence interval [CI], 1.6–473) and 10 March (OR, 8.0; 95% CI, 1.1–355) (table 1). Dinner on 9 March yielded an OR of 6.0 (95% CI, 0.7–276). In the unmatched case-control study, illness was associated with eating from the deli bar during lunch on 9 March (OR, 11.1; 95% CI,

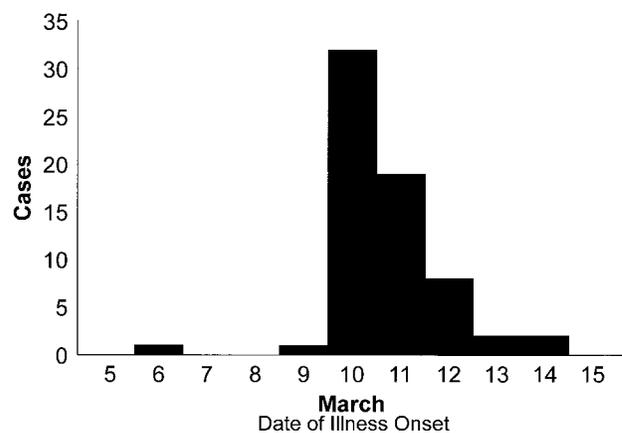


Figure 1. No. of cases of gastroenteritis, by onset date, among 65 students who participated in interviews at a university in Texas during March 1998.

Table 1. Risk factors for illness among subjects who ate in the main cafeteria at a university in Texas and participated in matched and unmatched case-control studies during March 1998.

Study, exposure	Ill	Well	Odds ratio	95% confidence interval	P
Matched^a					
Deli bar lunch on 3/9	11/28 (39)	1/29 (3)	11.0	1.6–473	<.01
Deli bar dinner on 3/9	7/27 (26)	2/29 (7)	6.0	0.7–276	.06
Deli bar lunch on 3/10	8/29 (28)	1/28 (4)	8.0	1.1–355	.02
Deli bar dinner on 3/10	2/29 (7)	2/28 (7)	1.0	0.01–79	.75
Unmatched^b					
Deli bar lunch on 3/9	18/30 (60)	12/101 (12)	11.1	3.9–32	<.001
Deli bar dinner on 3/9	7/18 (39)	5/61 (8)	7.1	1.6–33	<.01
Deli bar lunch on 3/10	13/29 (45)	12/96 (13)	5.7	2.0–16	<.001
Deli bar dinner on 3/10	4/16 (25)	4/44 (9)	3.3	0.6–20	.11
Deli bar lunch on 3/9, dinner on 3/9, or lunch on 3/10	28/36 (78)	20/116 (17)	16.8	6.2–47	<.001

NOTE. Data are no. exposed/total (%). Dates are given as month/day.

^a n = 29 for each group (ill and well).^b n = 36 for ill subjects; n = 136 for well subjects.

3.9–32) and 10 March (OR, 5.7; 95% CI, 2.0–16), as well as eating from the deli bar during dinner on 9 March (OR, 7.1; 95% CI, 1.6–33). The deli bar association was strengthened when all 3 meals were combined (OR, 16.8; 95% CI, 6.2–47).

Environmental investigation. At the university cafeteria, deli meats, cheeses, and condiments were prepared daily but were routinely held over for use the next day. Additional food items were added daily to partially depleted deli bar items, without discarding leftover food items. The deli bar containers were not cleaned on a daily basis. On 11 March, the City Health Department closed the deli bar to allay preliminary concerns that it might be the source of the outbreak.

One food handler initially chose to be suspended from her job rather than submit a stool specimen and be interviewed. This staff member was responsible for preparing the deli bar sandwiches during lunch on 9 March. When she later agreed to be interviewed, she reported slicing deli ham on 9 March for use during lunch and dinner that day and lunch the following day. She claimed to have worn gloves while slicing the ham and while serving sandwiches at the deli bar. She denied having any gastrointestinal illness during the outbreak period but reported that her infant had been sick with watery diarrhea since 7 March, 2 days before she prepared items for the implicated meals.

Laboratory investigation. Cultures of 62 stool specimens did not yield *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, *Listeria*, or *Yersinia* species or *Escherichia coli* O157:H7, *Bacillus cereus*, or *Staphylococcus aureus*. Of 18 fresh, whole stool specimens from ill students, 9 (50%) had evidence of NLVs by RT-PCR. Of the foods examined, only the ham sample was positive by RT-PCR for the presence of NLV. No inhibitors to PCR amplification were detected in the food samples tested. Analysis of the information obtained from sequencing the nested PCR amplicons showed absolute identity for 248 consecutive bases in the capsid gene, compared with the sequence obtained from

virus in the stool samples. NLV was also detected, by use of RT-PCR, in a stool sample collected on 23 March from the ill infant of the food handler who prepared deli sandwiches on 9 March; the sequence of the amplified product was identical to those of products from ill students and from the deli ham. All 10 stool samples (from 9 ill students and the ill infant) were positive by RT-PCR using the G2 primer set. The nucleotide sequences of 4 DNA products from a region of the capsid genome were identical and are consistent with classification in the Lordsdale virus cluster.

Discussion

Although molecular techniques have been used to detect NLV in shellfish [12], which concentrate microorganisms, it has been difficult to detect NLV in other foods because of the small number of virions and the presence of RT-PCR inhibitors. In this investigation, an epidemiologic assessment identified deli foods served at a university cafeteria as the likely source of the outbreak, and novel RT-PCR methods were able to confirm the presence of NLV with a unique sequence in specimens from ill students and on the surface of leftover deli ham. NLV with the identical sequence was also identified in fecal specimens from a food handler's infant, who had become ill a few days before the outbreak. The food handler, who sliced ham for all 3 implicated meals, denied illness but also chose to surrender her job rather than submit a stool specimen. A traceback of deli items served at the university identified no outbreaks at other institutions that received the same items, further suggesting that the source of contamination was a food handler at the university.

The clinical features of illness observed during this outbreak were generally consistent with those reported in previous NLV outbreaks: an incubation period of 24–48 h, illness of short duration, and a high percentage of patients with vomiting. The

finding of marked leukocytosis with band forms and a predominance of polymorphonuclear cells has also been reported previously in an NLV outbreak among college students [13]. Nevertheless, some features were distinctly uncharacteristic of NLV-induced illness. Fecal leukocytes were detected in 58% of stool specimens tested, occult blood was detected in 28%, and 5% of ill patients reported bloody stools. No other pathogens were isolated that might explain these findings, despite extensive culturing. Although it is impossible to completely exclude the presence of any other pathogen or cofactor, our findings suggest that certain strains of NLV may be capable of causing significant intestinal inflammation.

The local health department's initial hypothesis that food served from the deli bar was the probable source of infection led to its prompt closure and probably prevented further cases of gastroenteritis. Most public health departments recommend that food establishments exclude workers with acute gastroenteritis for 48–72 h after symptoms have resolved. Traditionally, adults have been considered to be infectious only in the first few days of infection, after which the development of antibodies may reduce viral shedding and transmissibility. However, studies of shedding have confirmed that viral material can be detected by immune electron microscopy >72 h after ingestion [14] and by ELISA for 1–2 weeks [15]. Studies have not evaluated stool specimens for viral genomic material beyond 2 weeks, and the duration of NLV shedding in infants is unknown. If the infant's illness of 7 March was indeed due to an NLV, our findings suggest that infants, at least, can shed virus for >2 weeks. As more is learned about the duration of NLV shedding, health officials may need to revise work-exclusion recommendations for food handlers, to prevent transmission. The adoption of a work policy that includes paid leave for ill employees with gastroenteritis would probably increase compliance with illness-related work-exclusion policies. Food handlers who are caretakers of persons with gastrointestinal illness should be encouraged to pay particular attention to personal hygiene, especially if the ill person is a diapered infant. Exclusion of everyone who cares for a family member with diarrhea is probably an unfeasible and draconian measure.

This is the first time molecular techniques have detected virus from a food item not likely to have been contaminated at the primary source. Given that stool and deli samples were tested in 2 laboratories in separate states, our findings are unlikely to reflect laboratory contamination. Use of genomic amplification and sequence analysis makes it possible to link clinical illness with an implicated food vehicle. Once RT-PCR procedures become established for deli meats and other foods, proper collection and storage of implicated food items will be necessary to enhance recovery of NLV. In future outbreaks, application of RT-PCR to characterize NLV in clinical and food samples

may enable us to identify new vehicles of infection and novel patterns of transmission.

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