Multistate Outbreak of Norwalk-like Virus Gastroenteritis Associated with a Common Caterer

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In February 2000, an outbreak of gastroenteritis occurred among employees of a car dealership in New York. The same meal was also supplied to 52 dealerships nationwide, and 13 states reported illness at dealerships where the banquet was served. A retrospective cohort study was conducted to identify risk factors associated with the illness. Stool samples were collected to detect Norwalk-like virus, and sera were drawn and tested for immunoglobulin A antibodies to the outbreak strain. By univariate analysis, illness was significantly associated with consumption of any of four salads served at the banquet (relative risk = 3.8, 95% confidence interval: 2.5, 5.6). Norwalk-like virus was detected by reverse transcription-polymerase chain reaction assay in 32 of 59 stool samples from eight states. Nucleotide sequences of a 213-base pair fragment from 16 stool specimens collected from cases in eight states were identical, confirming a common source outbreak. Two of 15 workers at caterer A had elevated immunoglobulin A titers to an antigenically related Norwalk-like virus strain. This study highlights the value of molecular techniques to complement classic epidemiologic methods in outbreak investigations and underscores the critical role of food handlers in the spread of foodborne disease associated with Norwalk-like virus. Am J Epidemiol 2001;154:1013–19.

Calicivirus; disease outbreaks; gastroenteritis; polymerase chain reaction

Norwalk-like viruses, formerly known as small round-structured viruses, are the most common cause of viral gastroenteritis among adults (1–3). Large outbreaks of gastroenteritis caused by Norwalk-like virus have been reported in a variety of settings. Norwalk-like viruses can be transmitted by fecally contaminated water, foods, or environmental surfaces, or directly from person to person (4). The role of Norwalk-like viruses in foodborne illness is well recognized. The typical route of contamination is either through food prepared by an ill food handler or through a bulk food supply contaminated at the production center. Salads, bakery products, ice, and shellfish have all been implicated as vehicles for infection (5–9).

Until recently, detection of Norwalk-like viruses required the use of electron microscopy or measurement of antibody response in paired serum specimens. However, these methods were of low sensitivity and did not allow the linking of multiple outbreaks together (9). The development of assays to detect Norwalk-like viruses in stool samples by reverse transcription-polymerase chain reaction (RT-PCR) has greatly improved the detection rate for Norwalk-like viruses (9–11). By using nucleotide sequence analysis of the amplification products, it is now possible to link outbreaks from a common source occurring in multiple locations and to examine the relatedness of cocirculating strains (12). In February 2000, a multistate gastroenteritis outbreak among car dealership employees was reported to the Centers for Disease Control and Prevention. An investigation was undertaken to determine the etiologic agent, vehicle of transmission, and the source of contamination. RT-PCR was used to identify the etiologic agent and perform sequence analysis of polymerase chain reaction products to confirm a common source for this outbreak. This report describes the epidemiologic, environmental, and laboratory investigations of this outbreak and illustrates the potential for widespread Norwalk-like virus infection due to a common source exposure.
MATERIALS AND METHODS

Background

On February 10, 2000, the Centers for Disease Control and Prevention received a report of an outbreak of gastroenteritis among employees of a car dealership in New York that was thought to be associated with a catered meal or “boxed banquet” served 2 days previously. The same meal was supplied to 52 dealerships in 27 states as a reward for high car sales (figure 1). Inquiries to other dealerships participating in the “boxed banquet” program revealed multiple cases of acute gastroenteritis among employees who attended the meal. The meals were shipped from a supplier in Ohio, who assembled them from foods prepared by four local caterers. Caterer A supplied the salads, condiments, dips, cheeses, and snacks. Caterer B provided the meats, including turkey, ham, and chicken. Caterer C prepared the desserts, and caterer D supplied the breads. Caterers A, C, and D shipped their packaged foods to caterer B, who then assembled the foods into a “boxed banquet.” The meal was shipped by overnight courier and scheduled to arrive at each dealership the day before consumption. Three meal shipments were sent out on February 7, 8, and 9. When early suspicions linked the gastroenteritis outbreak in New York to the “boxed banquets,” all dealerships that had meals scheduled to arrive on February 10 were instructed to dispose of the food by the dealership’s banquet coordinator, and the “boxed banquet” program was immediately discontinued pending investigation.

Epidemiologic investigation

Epidemiologic investigations were conducted in 14 states using a standardized questionnaire. A multistate retrospective cohort study was conducted by telephone or personal interviews of employees of the car dealerships where the meals were served. A case was defined as a person who had attended a “banquet dinner” at one of the dealerships and subsequently developed vomiting or diarrhea (three or more loose stools within a 24-hour period). All banquet attendees were eligible to participate in the investigation.

Health department staff in each state interviewed banquet attendees to determine whether they had been ill, reviewed their symptoms, and ascertained the foods they had consumed at the banquet. Interview questions covered a complete list of menu items served at the meal, symptoms, and the onset time and date of illness. Banquet attendees were also questioned regarding illness of any family members who had not eaten at the banquet. Data were entered and analyzed by use of Epi-Info version 6.04a software (13).

FIGURE 1. Multistate outbreak of gastroenteritis traced to a single food provider, United States, 2000.
Laboratory investigation

Bulk stool samples from 67 dealership employees in eight states were collected in clean, dry containers and refrigerated at 4°C before being tested. Specimens from 59 employees were tested for Norwalk-like virus by RT-PCR for amplification of a 213-base pair portion of the Norwalk-like virus RNA polymerase gene using a region B degenerate primer set (Fankhauser et al., unpublished data). In brief, reactions were performed with a set of degenerate primers including Norwalk-like virus 431 (tggac1agRggIccYaaYca), Norwalk-like virus 432 (tggac1cgYggIccYaaYca), Norwalk-like virus 433 (gaaYctcatccaYctgaacat), and Norwalk-like virus 434 (ggaYcgcatccaRcggaacat). Amplification products were confirmed by liquid hybridization assay, and positive samples were further characterized by nucleotide sequencing (14). Stool specimens were also tested for the presence of Salmonella, Shigella, Yersinia, Campylobacter, and Escherichia coli O157:H7.

Serum samples were collected from 20 car dealership employees in New York approximately 12 days after the onset of illness. Serum samples were also collected from 15 employees of caterer A and six employees of caterer B. The serum specimens were tested for immunoglobulin A antibody to Norwalk-like virus by using a recombinant-expressed Norwalk-like virus capsid protein in an enzyme immunoassay (15).

Samples of the implicated salads were collected from dealerships in eight states. Samples were tested at the Division of Environmental Health Sciences at the Johns Hopkins Bloomberg School of Public Health, where they were assayed for the presence of Norwalk-like viruses by RT-PCR according to methods previously described (16).

Environmental investigation

Food handlers from all four catering services were questioned regarding the procedures used in the preparation of food items and history of illness. Local health officials and investigators from the Centers for Disease Control and Prevention conducted an environmental assessment of caterer A to review kitchen facilities and the procedures used in the processing and handling of cooked and uncooked food. The inspectors also contacted other establishments that received food prepared by caterer A to inquire about cases of gastroenteritis among their patrons.

RESULTS

Epidemiologic investigation

Of the 753 banquet attendees surveyed, 333 (44 percent) met the case definition for gastrointestinal illness. Ill persons ranged in age from 3 to 89 years (median, 37 years) and included car dealership employees and family members who also attended the banquet. The symptoms most commonly reported by ill persons were diarrhea (78 percent), nausea (72 percent), abdominal cramping (76 percent), chills (54 percent), and vomiting (54 percent). The duration of illness ranged from 1 to 8 days (median, 2 days); in 86 percent of the cases, illness lasted for 4 days or less. Thirty-three attendees (10 percent) sought medical care, and three persons (1 percent) were hospitalized. Forty-five persons (13 percent) reported secondary gastrointestinal illness among household members.

The dates of illness onset for reported cases ranged from February 9 to 14, 2000, with the highest number of cases occurring on February 11, 2000 (figure 2). The meals were

shipped on 3 successive days, and the date of illness onset correlated with the date on which the meal was eaten; persons who consumed meals from the first shipment were the first group to become ill. This information yielded a minimal incubation period of 1 day for illness associated with each of the three banquet meals. The fewest number of cases were related to the third shipment, corresponding to discontinuation of the “boxed banquet” program on February 10 and prevention of food consumption at several dealerships.

The questionnaire was analyzed to identify exposures to food items having significant association with illness (table 1). The exposures were analyzed in four groups corresponding to the four food providers that prepared foods. Caterer A supplied the four salad items, and consumption of any of these four side salads was associated with illness (relative risk = 3.8, 95 percent confidence interval: 2.5, 5.6). Among the four side salads, the rotini pasta salad was most strongly associated with disease (relative risk = 3.0, 95 percent confidence interval: 2.4, 3.7). Consumption of condiments, breads, dips, and cheeses was also associated with illness in the crude analysis, but the risk did not persist after stratifying for consumption of any of the four side salads.

### Laboratory investigation

All stool specimens obtained from ill car dealership employees tested for the presence of bacterial pathogens were negative. Of 59 stool specimens collected from cases in nine states, 32 (54 percent) tested positive for Norwalk-like virus by RT-PCR. The polymerase chain reaction products from viral RNA of 16 specimens were sequenced to confirm a common outbreak strain, and all contained strains with an identical sequence belonging to Norwalk-like virus genogroup II.

Among the 20 car dealership employees from whom blood specimens were collected, 10 had eaten at the banquet and developed gastroenteritis, eight had eaten at the banquet but did not become ill, and two did not eat at the banquet and did not become ill. Among the car dealership employees who attended a banquet, immunoglobulin A antibody titers to Norwalk-like virus ranged from 2,100 to 50,800. Two employees of caterer A, both of whom had participated in preparing or packaging the meal, had elevated levels of immunoglobulin A antibody of 9,300 and 3,100, suggesting recent infection, compared with none of the six employees of caterer B (figure 3).

Samples of implicated salads that were tested were negative for Norwalk-like viruses by RT-PCR.

### Environmental investigation

All four caterers were inspected by a sanitaryian. Sanitation inspections of caterers B, C, and D were unremarkable and no violations were identified. An inspection of caterer A resulted in citations for 57 violations, and the business was closed by the local health department on February 16 for 2 days until these violations could be corrected.

In the preparation of salads at caterer A, pastas are boiled, drained, cooled, and then placed in large plastic bins. Other ingredients are added to the pasta, and the salad is mixed by hand, requiring food handlers to immerse their ungloved arms up to the elbow to mix all of the salad ingredients effectively. All caterer A employees denied any history of illness. No other establishments that received food from caterer A reported increased cases of gastroenteritis.

### DISCUSSION

This investigation confirmed that a catered meal prepared in Ohio and distributed to 52 car dealerships nationwide was responsible for multiple gastroenteritis outbreaks in 13 states, resulting in illness of at least 333 persons. The epidemiologic characteristics of this outbreak, including clinical symptoms, incubation period, and illness duration, were consistent with previous Norwalk-like virus outbreaks (1, 17). Consumption of any of four side salads produced by caterer A was strongly associated with illness. The point source for this outbreak was further supported by the identification of a Norwalk-like virus with a single genetic sequence identified among fecal specimens obtained from the four side salads.

### TABLE 1. Association of reported exposures with illness among car dealership employees in 14 US states, February 2000

<table>
<thead>
<tr>
<th>Caterer</th>
<th>Food items</th>
<th>Exposed</th>
<th>Unexposed</th>
<th>Relative risk</th>
<th>95% confidence interval</th>
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<tr>
<td></td>
<td></td>
<td>%</td>
<td>No.</td>
<td>Total no.*</td>
<td>%</td>
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<tr>
<td>A</td>
<td>Side salads</td>
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<td>310</td>
<td>585</td>
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<td></td>
<td>Rotini pasta salad</td>
<td>65</td>
<td>255</td>
<td>391</td>
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<td></td>
<td>Potato salad</td>
<td>52</td>
<td>233</td>
<td>444</td>
<td>33</td>
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<td></td>
<td>Bow-tie pasta salad</td>
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<td>110</td>
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<tr>
<td></td>
<td>Vegetable salad</td>
<td>64</td>
<td>120</td>
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<td>109</td>
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<td>Dips</td>
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<td>194</td>
<td>436</td>
<td>46</td>
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<td>Meats</td>
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<td>122</td>
<td>240</td>
<td>42</td>
</tr>
<tr>
<td>D</td>
<td>Breads</td>
<td>47</td>
<td>268</td>
<td>573</td>
<td>33</td>
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</table>

* The total number of responses varied according to the recall of the person queried.
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cases in eight states. While all employees of caterer A denied illness in the preceding week, two of 15 employees of caterer A had elevated titers of immunoglobulin A antibody to Norwalk-like virus, indicating a possible recent infection. Furthermore, caterer A was cited by health inspectors for multiple sanitary code violations and temporarily closed pending sanitary improvements. Although it is unknown whether better sanitary practices would have prevented this outbreak, the violations may indicate a decreased concern for hygiene.

Although we cannot exclude the possibility that a contaminated item was used to make the salad, our findings suggest that an ill food handler contaminated the salads during preparation. The possibility that contamination occurred during the processing of a salad item before salad preparation is unlikely, because there were no reports of increased cases of gastroenteritis among other persons served meals with similar ingredients by caterer A before the banquet meal.

Although stool samples were not available from caterer employees for viral testing during the outbreak, serum immunoglobulin A antibody levels to Norwalk-like virus were elevated in two caterer A employees. The higher titer of 9,300 was present in one of the food preparers. This titer is compatible with a recent infection. However, this result could indicate either infection with a different, but antigenically related, Norwalk-like virus strain, since strain characterization was not possible, or an infection that occurred several weeks previously.

The use of serology to detect immunoglobulin G antibody to Norwalk-like virus is well established (18). However, virus requires collection of an acute and convalescent serum sample with the first serum sample drawn within 5 days of symptom onset (19). Often, there is a lag time of several days after an outbreak is recognized before clinical specimens are collected to test for a viral etiology. In addition, it is possible for asymptomatic infection to occur with Norwalk-like virus infection, making it more difficult to know if a food handler is shedding the virus. Therefore, immunoglobulin A testing may be the only method for the late detection of a Norwalk-like virus infection as immunoglobulin A antibody peaks earlier than immunoglobulin G but for a shorter duration of time. However, methods for the testing of immunoglobulin A response to Norwalk-like virus infection are a relatively new technology, and the kinetics of immunoglobulin A response to Norwalk-like virus infection are still largely unknown. It is not possible to determine precisely when viral shedding would most likely occur solely on the basis of the immunoglobulin A titer. Thus, we cannot use serologic data alone, without evidence of viral shedding in the stool during food preparation, to implicate a single food handler as the cause of the outbreak.

We were unable to detect Norwalk-like virus in salad samples by using RT-PCR techniques. The detection of Norwalk-like viruses in foodborne outbreaks has been most successful when shellfish, which tends to concentrate microorganisms, is the vehicle of infection (20–22). Assays for the presence of Norwalk-like virus in other foods are less sensitive because of the potentially lower Norwalk-like virus titers present on the foodstuff and the presence of RT-PCR inhibitors.

The low infectious dose of Norwalk-like virus necessary to cause illness has resulted in outbreaks traced to a single
exposure source and affecting large numbers of people (5, 23–25). Food preparation involving the mixing of liquid ingredients by using bare hands has been shown to be an effective means of evenly contaminating food that will be widely distributed (5). In addition, previous research has demonstrated that viroms of viral gastroenteritis can be detected from handwashings of persons with contaminated hands (26).

Although the exact route of salad contamination could not be determined, the epidemiologic investigation and genetic analysis traced this multistate outbreak to the consumption of side salads produced from one source. Norwalk-like virus strains with the same sequence have been identified by routine surveillance in many different states over time in other outbreaks. This study provides new insights into the mechanisms by which such common strains can be spread throughout the United States. The ability to link outbreaks through sensitive detection methods and sequence analysis should encourage increased communication between local and state health departments to identify possible common vehicles or exposures in cases occurring in a temporally related cluster. These methods become increasingly important as contaminated foods produced in large quantities may be widely distributed and cause illness in geographically distinct areas, making it difficult to trace the source of outbreaks without the combined efforts of epidemiologic investigation and molecular analysis.

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REFERENCES


