OUTBREAK OF "NORWALK-LIKE VIRUS" GASTROENTERITIS ASSOCIATED WITH FOODHANDLERS: EVIDENCE OF PROLONGED VIRAL SHEDDING USING NEW DNA PRIMERS

"Norwalk-like viruses" (NLV) are the most common cause of foodborne illness in the United States accounting for an estimated 23 million cases of foodborne illness each year. Symptoms of NLV infection start within 2 days after exposure and include nausea, vomiting and diarrhea that usually last 24- 48 hours. The most common modes of transmission are contamination of food and water by feces and person-to-person transmission. Airborne spread via aerosolized vomitus also has been reported. 4

Foodhandlers are often associated with NLV transmission. Transmission has been documented from foodhandlers both before and after they resolve symptoms of NLV infection. Fecommendations differ on how to prevent the transmission of NLV by foodhandlers. In 1990, the Centers for Disease Control and Prevention (CDC) recommended restricting foodhandlers from preparing food for at least 2 days after resolution of gastrointestinal illness. However in 2001, the CDC issued new guidelines which stated that foodhandlers should maintain strict personal hygiene at all times but did not specify for how long foodhandlers should be removed from work. The 1999 Food Code issued by the Food and Drug Administration recommends restricting foodhandlers from preparing food only while symptomatic with vomiting or diarrhea. The National Restaurant Association and many state departments of health use this code as a model.

Experimental volunteer studies have documented Norwalk virus (the prototype NLV) shedding in stool by electron microscopy (up to 5 days), ¹² reverse transcriptase-polymerase chain reaction (RT-PCR) (6 days), ¹³ and enzyme-linked immunoassays (13 days)¹⁴ after exposure to Norwalk virus; maximal shedding occurs in the first 48 to 72 hours. Duration of shedding after resolution of gastrointestinal symptoms has not been systematically documented; this has important implications for how long to remove foodhandlers from preparing food. Based on the results of an investigation of a restaurant-associated outbreak of NLV, we were able to address this question.

Between March 27 and 31, 2000, the Acute Communicable Disease Control (ACDC) Unit received reports of 19 people, from 5 different groups, who became ill after eating at a restaurant in Los Angeles County (LAC). Interviews with 10 of the 19 restaurant patrons revealed that they had nausea, vomiting, or diarrhea within 24 to 48 hours after eating at this restaurant on either March 25 or 26. Based on these symptoms NLV was suspected. On March 31, during a restaurant inspection prompted by these reports, the environmental health investigator learned of 17 employees who also had gastrointestinal symptoms. Given the high risk of NLV transmission by foodhandlers, the restaurant was shut down and all employees had to be cleared by the health department before returning to work (i.e., they had to be free of gastrointestinal symptoms for at least 2 days).

ACDC investigated this outbreak to confirm the etiology of the outbreak and to determine the incidence of gastroenteritis among the employees during the last 2 weeks of March 2000. Results from this investigation allowed us to calculate the length of NLV shedding in stool after resolution of gastrointestinal symptoms.

METHODS

During April 1 to 4, employees of the restaurant were interviewed about symptoms of gastroenteritis for the last 2 weeks of March 2000. Questions included the type of symptoms, the date of onset, the duration of symptoms, and whether or not the employee worked while ill. In this outbreak, a case of acute gastroenteritis was defined to be 3 or more loose stools in 24 hours or vomiting. A possible case of gastroenteritis was 1 or 2 loose stools in 24 hours or nausea with either chills or fever.

The laboratory investigation consisted of collecting single stool samples from restaurant employees and patrons. The samples were tested for the presence of NLV using Region B RT-PCR primers at the Los Angeles County Public Health Laboratory. Selected positive RT-PCR amplicons were sequenced at an outside laboratory. The number of days shedding NLV after resolution of symptoms was calculated by subtracting the date of last gastrointestinal symptoms from the date of stool collection for positive specimens.

Epi-Info 6.04c (Centers for Disease Control and Prevention, Atlanta, GA, 1999) was used for data management and analysis.

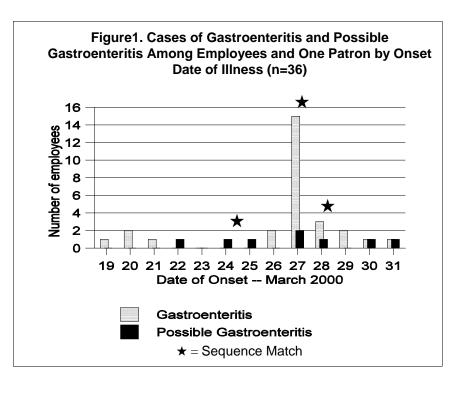
RESULTS

All 71 employees of Restaurant A were interviewed; 28 (39%) met the case definition of acute gastroenteritis and 7 (10%) were possible case-patients; the remaining 36 (51%) employees were asymptomatic. Figure 1 displays the epidemiologic curve of this outbreak. Half of the employees for whom information was available (16 of 32 employees) admitted to working while ill.

Laboratory investigation revealed that 12 (57%) of 21 employees who met the case definition for acute gastroenteritis and who submitted stool samples tested positive for NLV. In contrast, only 1 (14%) of 7 possible case-patients tested positive for NLV and 4 (20%) of 20 asymptomatic employees who submitted stool samples also tested positive for NLV. Three samples (from a cook, a waitress, and a patron with symptom onset dates 3/24, 3/27, and 3/28, respectively) were submitted for DNA sequencing and all 3 were identical. Though only 2 of 8 case-patients had NLV in their stool 0 to 2 days after resolution of symptoms, 10 (77%) of 13 continued to shed NLV in their stool 3-11 days after resolution of gastrointestinal symptoms; one case-patient was still shedding at 11 days.

DISCUSSION

NLV was suspected as the cause of this outbreak among restaurant employees and patrons based on clinical symptoms; this was confirmed by the laboratory results. epidemiologic curve (Figure 1) shows that NLV may have been transmitted at a low level among restaurant employees for almost a week before the larger outbreak among restaurant employees and patrons. Patrons were exposed March 25-26 and the majority of employees were probably exposed at that time since most of the ill



employees became ill between March 26-28, the same time as the restaurant patrons. We were unable to identify a single food item as the vehicle of transmission for the outbreak (data not shown). A cook who was ill with mild gastrointestinal symptoms for only one day on March 24 worked on March 25 and 26. Since the NLV in his stool was genetically identical to the virus in the stool of a patron who ate at the restaurant on March 26, the cook could have been the source of the large outbreak. However, more than one foodhandler may have been shedding and transmitting NLV during the weekend of March 25 to 26 leading to contamination of multiple food items.

Using the Region B RT-PCR primers, we found that the majority (77%) of foodhandlers shed NLV more than 3 days after resolution of gastrointestinal symptoms, with a maximum duration of shedding tested at 11 days after resolution of symptoms. What was unusual was that only 2 of 8 stools tested positive for NLV within 2 days after resolution of symptoms. Previous experimental studies have documented the highest rate of shedding in the first two days after exposure to virus. However, those studies used actual Norwalk virus while the agent in our outbreak was a Norwalk-like virus; it is possible that there are clinical differences in how quickly humans stop shedding different NLV strains.

Since stool samples were taken at only one point in time our estimates of the duration of shedding must be regarded as minimums since employees may have continued to shed NLV after we

collected their stool sample. Furthermore, the number of employees infected with NLV is probably underestimated since some may have stopped shedding NLV in their stool before the samples were collected.

Current recommendations from the CDC suggest to preferentially collect stool specimens within the first 48-72 hours after patients become ill, and from patients with watery or unformed stool. However, we found that persons who had vomiting but no diarrhea (data not shown) were as likely to have a positive stool result as persons who had diarrhea. Our study indicates that the Region B RT-PCR primers may be sensitive enough to detect NLV in stool for up to 11 days after resolution of symptoms and from case-patients who have vomiting but not diarrhea. These findings should encourage the collection of stool samples longer than previously thought and from a wider variety of patients. Working with a local public health laboratory is essential to identify NLV. Tests for NLV are not commonly available in commercial laboratories.

To decide how long foodhandlers should be restricted from preparing food, better studies are needed to assess the risk of shedding NLV and transmission of the virus. These studies should include epidemiologic findings documenting both post-symptomatic and asymptomatic transmission of NLV by foodhandlers. RT-PCR is a sensitive and powerful tool, though in real world applications, it is assumed that at least 100-10,000 viral particles/ml of stool are required for a positive test. However, since the estimated infective dose of NLV is only 10-100 particles, a positive test result suggests that there are sufficient viral particles for transmission of the disease if a foodhandler has poor hygiene. RT-PCR tests need to be developed that quantify the decrease in the viral load shedding after resolution of symptoms to quantify transmission risk. Region B RT-PCR primers only tests for the presence of NLV RNA; it still needs to be shown that the RNA is infectious, i.e., in an intact, infectious virion.

Given that half of the employees surveyed admitted to working while ill, we recommend enforcing the FDA Food Code Guidelines to restrict foodhandlers from working while symptomatic. We also suggest expanding the FDA recommendations to restrict foodhandlers for at least 2 days after gastrointestinal symptoms resolve according to the 1990 CDC recommendations. If the foodhandler no longer has diarrhea and adheres to adequate handwashing and gloving, as called for in the FDA Food Code, then there may be no need to restrict the foodhandler from work for longer than 48 hours even if they are still shedding NLV.

Finally, paid sick leave, which may not be commonly offered to hourly employees in the restaurant industry, may reduce the number of employees who work while ill and may have avoided this outbreak.

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