Multi-State Outbreaks of Acute Gastroenteritis Traced to Fecal-Contaminated Oysters Harvested in Louisiana

Debra E. Berg,1 Melvin A. Kohn,2 Thomas A. Farley,3 and Louise M. McFarland

Norwalk-like viruses (NLVs), or small round structured viruses (SRSVs), are members of the family Caliciviridae and are known to cause acute gastroenteritis associated with eating raw shellfish, especially oysters [1]. The viruses are highly infectious, and because only a small inoculum is required to cause illness, attack rates generally range from 50%–90% [2]. Although NLVs were first described as a pathogen in Norwalk, Ohio, in 1968, outbreaks associated with eating shellfish were not described until 1976 [3, 4]. In England and Australia, investigations have documented that sewage-contaminated oysters could be the source of outbreaks of NLV-related gastroenteritis [4–7] (table 1).

In the United States, the first documented outbreak of NLV-related gastroenteritis following raw oyster consumption occurred in Florida in January 1980 [8]. Since 1993, Louisiana has experienced three large NLV-related gastroenteritis outbreaks associated with the consumption of raw oysters [9–11]. Each incident illustrated how sewage discharge can cause NLV outbreaks and that prevention of these outbreaks presents a major public health challenge to public health officials.

Investigation of Oyster-Related Outbreaks of Viral Gastroenteritis

The epidemiologic tools used in the investigation of oyster-related gastroenteritis outbreaks include case finding, environmental investigation, and laboratory studies. Case finding entails working with local health departments, state epidemiologists, the news media, and the public to identify and interview individuals who become ill after ingesting oysters suspected of being contaminated. Case identification is often difficult because oysters are shipped to different places; thus, it is hard to accurately determine who ate oysters and where they ate them [12].

Environmental investigation of oyster-related gastroenteritis outbreaks includes oyster trace-back activities, interviews with persons in the oyster industry, and visits to possible sites of contaminated oysters. The goal of oyster trace-back activities is to determine, in reverse order, the path that the contaminated oyster has taken from the oyster bed to the ill consumer. Hence, a likely source of contamination can be determined. In general, there are at least three points on this path—harvesters, wholesalers, and retailers—and contamination can occur at any point. The site of contamination should be an individual location in this pathway that is shared by oysters eaten by most of the ill consumers.

Under the law, records are kept on each batch of oysters. In Louisiana, for example, oysters are transported in sacks that contain ~200 oysters, and state regulations mandate that each sack be tagged with the name of the harvester and the date and location of the harvest. Wholesalers are required to keep records with the tagging information for the sacks that they have sold, and retailers are required to keep the tags for 60 days. The trace-back investigation is dependent on the accuracy and availability of tagging information and on the cooperation of the oyster industry.

Because diagnosis of viral infection depends upon timely specimen collection, stool samples need to be collected for viral diagnosis as soon as an outbreak is identified. Electron microscopy can be used to examine stool samples for the presence of NLVs, which often are described by microscopists as SRSVs.
Table 1. Reported oyster-related outbreaks due to Norwalk-like viruses and in which oyster contamination occurred prior to harvest.

<table>
<thead>
<tr>
<th>Location</th>
<th>Month, year</th>
<th>No. ill</th>
<th>Laboratory confirmation in</th>
<th>Identified source(s) of sewage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louisiana</td>
<td>November 1993</td>
<td>190</td>
<td>EM, RT-PCR, S</td>
<td>Oyster harvesters</td>
<td>[9]</td>
</tr>
<tr>
<td>Florida</td>
<td>November 1993</td>
<td>30</td>
<td>EM, RT-PCR, ND</td>
<td>Oyster harvesters</td>
<td>[22]</td>
</tr>
<tr>
<td>Florida</td>
<td>January 1995</td>
<td>70</td>
<td>EM, RT-PCR, ND</td>
<td>Oyster harvesters, recreational boaters</td>
<td>[23]</td>
</tr>
<tr>
<td>Louisiana</td>
<td>February 1996</td>
<td>72</td>
<td>EM, RT-PCR, S</td>
<td>Oil rig, oyster harvesters</td>
<td>[10]</td>
</tr>
</tbody>
</table>

NOTE: Outbreaks in which only 1 party was identified were excluded. EM = electron microscopy, S = serologies, ND = not done, RT-PCR = reverse transcriptase–polymerase chain reaction.

More sensitive detection of NLV is possible with recently developed reverse transcriptase–polymerase chain reaction (RT-PCR) methods. Determination of the nucleotide sequence of the PCR products allows for further classification of NLV strains into one of several genetic clusters or genogroups, which sometimes can be used to confirm that different groups of ill persons are or are not linked to a common source of contamination. Diagnosis of NLV infection is also possible by testing paired serum samples for IgG or IgA antibodies to recombinant capsid proteins from Norwalk virus (NV) or other NLV strains by enzyme immunoassay.

Laboratory studies of oysters and water samples from harvest areas also can be useful in outbreak investigations. Viral genomic material from oysters can be amplified using RT-PCR and then sequenced [13–16]. The finding of an identical sequence in stools from ill persons and in the implicated oysters can be used to confirm the epidemiologic evidence linking ill persons and specific lots of oysters.

Water samples from harvesting areas are generally tested for fecal coliforms as a crude indicator of fecal contamination. However, there is only a weak correlation between bacterial indicators in oyster-harvesting waters and the presence of enteric viruses in oysters [17, 18]. An accurate marker to detect viral contamination of oysters is not commercially available; however, experimental studies using PCR have detected human adenoviruses in shellfish [19].

Oyster harvesting in the southeastern part of the United States is an important industry and source of employment. Louisiana produces ~520 million oysters each year. About 60% of oysters in the Gulf Coast region come from Louisiana waters, and about one-quarter of the nation’s supply is provided by Louisiana [20]. Between September and April, an estimated 2000 harvesters dredge Louisiana’s oyster beds, and ~4800 people are employed in this labor-intensive industry.

Outbreak one: November 1993. In November 1993, the Louisiana Department of Health and Hospitals received reports of groups of persons in six states who became ill with gastroenteritis after eating raw oysters. An extensive investigation in Louisiana was conducted to determine the characteristics and cause of the outbreak [9]. Seventy ill persons in Louisiana and 120 ill persons from five other states had gastroenteritis related to oyster consumption (figure 1). The oysters consumed were traced to a single harvest area in the Mississippi Sound called Grand Pass and Cabbage Reef. This area was closed for 30 days beginning the day after the first reports.

Of 14 serum pairs from ill persons, 11 (79%) had a ≥4-fold increase in levels of IgG antibody to NLV. SRSVs were found by electron microscopy in stool samples from all 12 ill persons tested. RT-PCR testing was also positive for a NV in all 12 samples. Nucleotide sequencing of the RT-PCR products from all 8 samples sequenced contained identical sequences with 74% identity with the prototype NV. Oyster samples were initially negative for NLV by RT-PCR; however, virus was later detected in the samples.

Investigation of 26 oyster harvesters who worked in the implicated waterways revealed that most of them (85%) discharged their sewage directly into the water. Six harvesters from four boats that worked in Grand Pass and Cabbage Reef reported being ill with vomiting or diarrhea during the month of the outbreaks. Two of the harvesters said that they were ill with vomiting and diarrhea while working in the heavily harvested part of the Grand Pass–Cabbage Reef area and that they disposed of their vomitus and feces overboard. The 2 men denied eating raw oysters. Serum samples from 2 non-ill and 2 ill harvesters had high levels (>5000) of IgA antibody to NV, suggesting recent infection.

The investigators concluded that the outbreak was caused by contamination of oysters in the oyster beds, probably by stool from ≥1 ill harvester. Their calculations indicated that because oysters are efficient at filtering and concentrating enteric viruses and human stools contain millions of virus particles, the stool from 1 ill person would be sufficient to contaminate an oyster bed 1 km long by 100 m wide by 2 m deep. This is about half the size of the waterways described above.

This investigation was the first to demonstrate overboard disposal of sewage from ≥1 ill harvester as a likely cause of an oyster-related NLV outbreak. In addition, the new RT-PCR...
tools were used to confirm the epidemiologic linkage of cases to a common source of contamination. The investigators recommended that regulations prohibiting the overboard discharge of sewage in oyster-harvesting areas be more strictly enforced. It was advised, as a way of discouraging this practice, that efforts be made to educate oyster harvesters about the risks that overboard sewage disposal pose to their livelihood.

Outbreak two: February 1996. The second oyster-related outbreak occurred in February 1996, during which time oysters again were thought to be contaminated in oyster beds, but the source of contamination was a malfunctioning sewage disposal system on an oil rig [10]. Seventy-five persons from four states developed gastroenteritis from eating raw oysters harvested from Louisiana. SRSVs were found in stools from individuals who had consumed oysters at several different social gatherings. The RT-PCR products from stool samples had the same genetic sequence.

The trace-back investigation revealed that the implicated oysters had been harvested in South Black Bay (figure 2). Nine harvesters were known to have harvested around South Black Bay, and tags from oyster sacks were available from 8 of the 9 harvesters. Five (63%) of 8 harvesters worked around Stone Island in South Black Bay, and the other 3 worked in areas other than Stone Island. Harvesters linked to oysters that caused the outbreaks were 4.4 times more likely than those not associated with the outbreak to have harvested around Stone Island (5/8 [63%] vs. 3/21 [14%]; relative risk: 4.4; 95% confidence interval: 1.4, 4.2)

A visit to Stone Island revealed a functioning oil rig that had among its 50 employees 8 who admitted to having diarrhea or vomiting between January and March. Four or 5 employees with gastrointestinal symptoms had elevated titers for NV, compared with 0 of 2 employees with nongastrointestinal symptoms.

In addition to the sick employees, the oil rig also had a malfunctioning sewage facility that discharged cloudy and feculent effluent directly into the surrounding waters. Staff from the oil rig reported that oyster harvesters routinely worked within a prohibited zone surrounding the oil rig.

This outbreak also demonstrated that oysters could be contaminated in oyster beds. The faulty sewage system on the oil rig might have contributed to the problem. Even if the sewage system was properly functioning, it is unclear if this would have prevented the outbreak. Chlorination has not been shown to adequately kill NLVs [21]. Furthermore, the investigators could not rule out harvester contamination from harvesters directly
dumping their waste overboard, since this practice was still taking place during this outbreak.

The recommendations based on the investigation included the following: Prohibit oyster harvesting within one-half mile of oil production facilities, implement stricter regulation of sewage treatment systems on oil facilities, require toilets on harvest boats, and have regular inspection and waste acceptance stations on docks.

**Outbreak three: December 1996.** Between 30 December 1996 and 7 January 1997, the Louisiana Department of Health and Hospitals determined that 153 persons from five states had become ill after eating oysters harvested from Louisiana waterways [11]. SRSVs were found by direct electron microscopy in fecal samples from 8 of 11 ill attendees of six unrelated social gatherings. Genetic sequencing of RT-PCR products from stool samples of ill consumers in 6 clusters showed three unique genetic sequences. Each sequence was associated with a different harvest site (table 2). Sequence 1 was associated with 3 groups of individuals who had eaten oysters harvested from Black Bay. Sequence 2 was associated with 2 groups who had eaten oysters harvested from Lake Machias. Sequence 3 was associated with 1 group who had eaten oysters harvested from California Bay. SRSVs were detected in oysters, but genetic sequencing could not be conducted because of technical reasons.

Twenty oyster harvesters were identified as having harvested in the area. Fourteen of the 20 were interviewed. Of eight oyster-harvesting boats inspected, seven had inadequate sewage collection and disposal systems.

Public health measures to control the outbreak included closing eight waterways, which was 60% of available public harvesting waters in Louisiana; enforcing stricter rules for the usage of toilets on boats; and creating waste acceptance stations at docks. One month after closure, the waterways were reopened, and no additional cases of oyster-associated gastroenteritis were reported.

The third outbreak differed from the other two because more than one source (i.e., three different harvest waterways) were identified, and each waterway was associated with a unique Norwalk virus strain. The data suggested the possibility that several oyster harvesters infected with unrelated NV strains might have discharged sewage in different harvest sites. This outbreak also documented that overboard discharge was still occurring despite the previous two outbreaks and despite a public health campaign to discourage oyster harvesters from dumping their waste overboard.

**Discussion**

Multi-state oyster-related outbreaks due to NLV are a continuing problem. Table 1 illustrates eight outbreaks in which >1 cluster has been identified and in which oyster contamination occurred prior to harvest. All 8 outbreaks identified human sewage as a source of oyster contamination. Five large outbreaks occurred in the United States between 1993 and 1996, compared with none occurring prior to 1990. Although this increase may be due to better detection capabilities and increased consumer awareness, environmental “bad habits,” such as sewage discharge directly into oyster-harvesting waters, has been on-going.

Florida experienced two outbreaks in which >1 cluster of people was involved [22, 23]. The outbreak in 1993 occurred during the same time period as the Louisiana outbreak. However, the NLV nucleotide sequence isolated in the Florida outbreak had a different pattern than that isolated in the Louisiana outbreak. As in the Louisiana episodes, oyster contamination was thought to occur prior to harvest. During the January 1995 Florida outbreak, several oyster harvesters admitted to being sick and were noted to use improper waste-disposal methods [24]. Recreational and commercial boaters were also in the area [23].

The practice of dumping sewage overboard continues in Louisiana despite good evidence that such disposal by oyster harvesters into oyster bed waters was the most likely source of sewage in at least two of the NLV gastroenteritis outbreaks. Changing this behavior seems to be the most likely way to prevent future oyster-related NLV outbreaks.

**Table 2.** Oyster harvest sites in Louisiana associated with 3 unique genetic sequences isolated from stool samples from case-patients, December 1996.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Harvest site</th>
<th>Viral sequencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Black Bay</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>Black Bay</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>Black Bay</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>Lake Machias</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>Lake Machias</td>
<td>2</td>
</tr>
<tr>
<td>F</td>
<td>California Bay</td>
<td>3</td>
</tr>
</tbody>
</table>

a Viral sequence 1 was identified as Snow Mountain Agent; sequence 2 was identified as Lordsdale virus, and sequence 3 was identified as Toronto virus.
tainer and make facilities for emptying them widely available, but compliance is impossible to implement without changing attitudes within the oyster industry. Despite the Louisiana outbreaks, it appears that many oyster harvesters are not convinced that overboard discharge is responsible for the contamination. Their suspicion is fueled by the economic competition between harvesters, by seafood and other food industries, and by the threat of government regulation. Such practices might be influenced by enlisting the help of elected officials from the areas where oyster harvesting is an important industry and having local enforcement agencies that interact with the harvesters on a daily basis.

In 1997, a group of Louisiana state and federal officials convened to focus attention on these issues. This group generated a report with a clear set of recommendations that was presented to the Interstate Shellfish Sanitation Commission (ISSC), the voluntary national group that sets standards and policies for regulation of the shellfish industry [26]. Subsequently, a resolution was passed by the ISSC acknowledging the association between oyster-related outbreaks and the overboard disposal of sewage [27]. Responsibility of implementing and enforcing effective controls relating to sewage discharge from harvest vessels was left to the states. Louisiana has since enhanced inspections of sewage disposal facilities on oyster-harvesting boats, but it is unclear if this activity will prevent overboard sewage discharge.

At present, the most direct approach to preventing these outbreaks is to prevent the oysters from becoming contaminated in the first place. However, if sensitive assays for NLV in oyster meats and oyster waters were available, public health officials might be afforded the opportunity to monitor oysters and oyster beds and close beds that have become contaminated. Such assays have yet to be developed.

If environmental issues predisposing to an outbreak could be clearly identified, it might be feasible to close oyster beds during high-risk periods. Once the virus is deposited into the oyster beds, low water flow, lower water temperature, and decreased water salinity may increase the likelihood that an oyster will filter a virus particle and retain it [28]. During the 1993 outbreak in Louisiana, tidal flow in the implicated oyster beds was below normal [9]. Similarly, low tides occurred just prior to both of the 1996 outbreaks, and a fall in water temperature and salinity was documented just before the December 1996 outbreak [10, 11]. Before such an approach can be tested, more thorough study of these factors is needed to clearly define these environmental conditions.

Summary

In summary, oyster-related outbreaks due to disposal of sewage into oyster waters continue to be a problem. The small infectious dose of NLV, the large quantity of virus particles in stool, and the ability of oysters to concentrate virus particles suggest that oyster-related outbreaks will continue unless strong control measures are established. Efforts to halt improper sewage disposal, including overboard sewage discharge, must be undertaken if future outbreaks are to be prevented.

Acknowledgments

We thank Susan Wilson for her careful data collection during all three outbreaks; Kenneth Hemphill, James Antoon, John Veazey, and Brett Koonse for the information supplied following the outbreaks; and Steve Monroe for his thoughtful review of the manuscript.

References


