Norwalk-like Viruses Associated With A Gastroenteritis Outbreak Following Oyster Consumption

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ABSTRACT

In late October 1991, an outbreak of gastroenteritis, following the consumption of raw oysters involving more than 200 people, was reported in five locations in Quebec, Canada. Bacteriological analysis of the oysters involved indicated low levels of fecal coliforms, but direct electron microscopy of stool samples obtained from two people involved in the outbreak revealed that both contained 27-34 nm, small, round Norwalk-like viruses. Immunoelectron microscopy, using acute sera obtained from these individuals and convalescent serum from another person related to the outbreak, revealed antibody coatings on these Norwalk-like viruses with all three sera. Solid-phase immunoelectron microscopy demonstrated that these viruses were also antigenically similar or related to a Norwalk-like virus isolated as the cause of an outbreak, revealed antibody coatings on these Norwalk-like viruses with all three sera. Solid-phase immunoelectron microscopy demonstrated that these viruses were also antigenically similar or related to a Norwalk-like virus isolated as the cause of the outbreak due to the consumption of raw oysters in a home for the aged between December 1988 and January 1989 in Thunder Bay, Ontario. From these findings and the symptoms of the illness, the Norwalk-like virus was considered as the causal agent of the outbreak due to the consumption of contaminated oysters. How the oysters became contaminated was not determined. Oysters harvested from the areas initially thought to have been the origin of the implicated shellfish were tested for the presence of viral fecal indicators using tissue culture and electron microscopy with negative results. It is most likely that the implicated lot also contained oysters harvested from another area, also open, but which was downstream from an identified source of human fecal contamination.

Outbreaks of gastroenteritis following the consumption of raw or partially cooked shellfish often have been associated with bacterial agents (6,19), nonbacterial gastroenteritis linked to shellfish has only been reported in the United Kingdom (1,4), Australia (12,15), and the United States (5,14). The illnesses in these incidents have been attributed to a 27-34 nm viral agent described as a Norwalk-like or Norwalk virus, first described in 1972 by Kapikian et al. (9). In late October 1991, there were reports of illness in more than 200 people related to the consumption of fresh oysters in five different locations in Quebec, Canada. Symptoms of the illness were nausea, vomiting, abdominal cramps, and diarrhea, occurring 24 to 36 h after consumption of the oysters. Four persons presented themselves to hospital but were released after observation.

There have been gastroenteritis outbreaks reported in Canada caused by a small, round 27-34 nm virus, morphologically similar and serologically related to the Norwalk agent, but the major reported outbreaks have all occurred in institutions (10,13) except for one outbreak in a hotel involving 386 people (18). As far as the authors are aware, this is the first report of a shellfish related gastroenteritis outbreak in Canada linked to a Norwalk-like virus.

MATERIALS AND METHODS

Oysters

Since the oysters associated with the outbreak were assumed to have been harvested from oyster-harvesting areas in Buctouche Bay and the connecting Richibucto River in northern New Brunswick, shellfish from this area were obtained for analysis. Batches of 15 oysters were shucked and washed and the contents homogenized. The homogenates were treated using a technique described by Speirs et al. (19) in order to recover and concentrate any viruses present. The concentrates were tested in two ways: (i) samples of the homogenate were cultured using four different cell lines in an effort to determine whether viral fecal indicators such as poliovirus or rotavirus were present, or (ii) samples of the concentrate were examined by direct electron microscopy (DEM) and by immunoelectron microscopy (IEM) as described below.

Stool and serum specimens

Stool and serum samples were obtained from two persons involved in the outbreak. Three consecutive stool samples were obtained from a 50-year-old male and were labelled QO1F, A, B, and C. Three fecal samples were also obtained from a 30-year-old female and were labelled QQ2F, A, B, and C, respectively. Serum samples were also obtained from these patients and were designated QO1S and QO2S. Since these samples were obtained 2-3 d after the onset of the illness, they are considered to be acute serum samples. Another serum sample was obtained from a 25-year-old oyster handler who had worked in the plant where all of the oysters involved in the outbreak were processed. He had eaten some oysters and had become ill but did not report it until some time later. This sample can be considered as convalescent serum and was designated QO3S.
RESULTS AND DISCUSSION

No viruses could be detected when the concentrates from the oyster homogenates were incubated with the different cell cultures. However, these results only indicated that the batches of oysters tested appeared to be free from viral indicators of fecal contamination such as poliovirus or rotavirus. DEM and IEM of the oyster concentrates were also negative; no viral structures were observed. These tests indicated that oysters harvested from open-harvesting beds in northern New Brunswick, the same area where the oysters involved in the outbreak were reported to have originated, showed no evidence of viral fecal contamination. We were unable to obtain samples of oysters directly involved in the outbreak.

DEM examination of fecal concentrates from stool specimens QOIF and QO2F revealed small, round 30-34 nm Norwalk-like virus particles in both samples (Fig. 1), although more particles were detected in the A sample of QOIF than in a similar sample of QO2F. Few particles were observed in the B samples of the stool specimens and none in the C samples. These results might be expected since this type of virus is shed into the feces only for a short period of time (7). Indeed, we were fortunate to obtain stool samples early enough (2 d after onset of illness), while viral shedding was still taking place, a rare occurrence in this type of outbreak.

The presence of Norwalk-like agents in the stools of these patients and the results of the microbiological analysis of the oysters carried out by the Ministère de l’Agriculture, des Pêcheries et de l’Alimentation de Québec (MAPAQ) (Table 1) showed low bacterial counts indicated that the outbreaks were not due to a bacterial agent, and therefore, a viral agent may be considered as the probable cause of the gastroenteritis.

To further confirm this hypothesis, when IEM was performed on the fecal concentrates QOIF and QO2F using acute serum QO1S, aggregates of 29-34 nm Norwalk-like viruses were observed as before but were coated with antibody which covered the viruses and agglutinated them (Fig. 2). This type of virus was present in the stools of patients with gastroenteritis caused by a Norwalk-like virus which occurred in a home for the aged in Thunder Bay, Ontario, between December 1988 and January 1989 (13).

TABLE 1. Microbiological analyses of oyster samples taken from different sources linked to the gastroenteritis outbreak*

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of samples</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic mesophiles</td>
<td>49</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;-10&lt;sup&gt;5&lt;/sup&gt; CFU/g for 49 samples</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>77</td>
<td>&lt;12 CFU/g for 8 samples</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>49</td>
<td>Negative</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>40</td>
<td>Negative</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>35</td>
<td>Negative</td>
</tr>
<tr>
<td>Vibrio vulnificus</td>
<td>35</td>
<td>Negative</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>78</td>
<td>10 to 2.5 x 10&lt;sup&gt;5&lt;/sup&gt; for 43 samples</td>
</tr>
<tr>
<td>Salmonella</td>
<td>49</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* These analyses were carried out by the Ministère de l’Agriculture, des Pêcheries et de l’Alimentation de Québec (MAPAQ), using oysters from the outbreak and samples from the retailers and wholesalers who supplied oysters to locations where the outbreaks occurred.
Figure 2. Micrograph of stool concentrate after treatment overnight at 4°C with acute serum. Sample negatively stained. Note fuzzy coating of antibodies surrounding viral particles and linking them together. Bar = 100 nm.

of reaction of antibodies with viruses has been described by several researchers (8,9,16) particularly in IEM studies of noncultivable viruses of the Norwalk group. The fuzzy antibody coating was much less noticeable when serum QO2S was used against the stool concentrates. Since both samples are acute sera, the antibody titer would normally be low and the reaction obtained with QO2S serum might be considered more normal. The heavier antibody coating obtained with QO1S serum might be explained by the fact that stool concentrate QO1F was obtained from a 50-year-old patient, and Kapikian et al. (9) have reported that the antibody titer to viruses of the Norwalk group increases with age and reaches its maximum in North America in individuals 50 and older. The more normal antibody response of the acute serum from patient QO2 can be related to her age, 30.

Further confirmation that the Norwalk-like virus was the probable causal agent in the outbreak was obtained when the convalescent serum QO3S from the oyster handler produced a fuzzy antibody coating on 23-34 nm viruses in both QO1F and QO2F fecal concentrates.

With the SPIEMGIC-TLM, it was observed that the Norwalk-like viruses found in QO1F formed immune complexes with the convalescent serum (#15C), as can be seen by the number of gold particles attached to the viruses (Fig. 3), and to a much lesser extent using acute serum (#15A) (Fig. 4) from a patient involved in a gastroenteritis outbreak in Thunder Bay, Ontario. These results indicate that the Norwalk-like virus observed in the stool samples from the Quebec outbreak is antigenically related or similar to the Norwalk-like virus isolated as the causal agent of the outbreak of gastroenteritis in a home for the aged in Thunder Bay. All these results strongly suggest that the Norwalk-like virus observed in the stools of individuals involved in the gastroenteritis outbreak is most probably the causal agent.

Although the Norwalk-like virus observed cannot be positively identified, the epidemiology of the outbreak fits very closely to the criteria set out by Kapikian and Chanock (7) to be symptomatic of a Norwalk infection: (i) vomiting occurred in more than 50% of the cases; (ii) duration of illness ranged from 12 to 60 h; and (iii) there was an incubation period of 24 to 48 h. The fourth criterion, the absence of bacterial or parasitic pathogens, was not fully met, but the results of the bacteriological analysis (Table 1) do not point to a bacterial agent being the cause of this outbreak. The
symptoms of the illness that occurred in this outbreak strongly suggest that the Norwalk-like virus found in the stool samples can probably be considered in the Norwalk virus group.

How the oysters became contaminated was not clear at first. Extensive investigations by the Department of Fisheries and Oceans Canada suggest that oysters harvested from an oyster bed other than the open ones in Buctouche Bay were mislabeled and subsequently contaminated the oysters that were consumed. Our tests with homogenates from oysters harvested from the initially implicated beds indicated that fecal pollution (shown by the presence of poliovirus or rotavirus) was not present. Unfortunately, samples of the oysters consumed in the outbreak could not be obtained. Although the actual source of oysters, not identified until some months following the outbreak, was an open-harvesting bed, it was downstream from an inadvertent sewage overflow which could have been the source of human fecal contamination. This bears some resemblance to the cause of a larger outbreak in Australia, in which oysters harvested from a closed area were mixed with those from an open area (15).

This study was concerned with the demonstration that a Norwalk-like virus was probably the causal agent of a gastroenteritis outbreak in Quebec following the consumption of contaminated raw shellfish. Besides this type of agent, hepatitis A virus has been shown to occur in uncooked oysters (6,19). Other viruses may also be transmitted by shellfish due to fecal contamination, but their detection depends on the development of suitable viral diagnostic techniques.

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REFERENCES