Internationally Distributed Frozen Oyster Meat Causing Multiple Outbreaks of Norovirus Infection in Australia

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Background. Between November 2003 and January 2004, outbreaks of norovirus in 3 Australian jurisdictions involving 83 cases of illness were associated with imported oyster meat.

Methods. Cohort studies were conducted in 2 jurisdictions to identify relative risks of illness for the consumption of oysters. A case series was conducted in the third jurisdiction.

Results. The cohort studies conducted in the first 2 jurisdictions identified relative risks of illness of 17 (95% confidence interval, 5–51) and 35 (95% confidence interval, 5–243), respectively, for the consumption of oysters. Multiple strains of norovirus were detected in fecal specimens from 8 of 14 patients and in 1 of the 3 batches of implicated oyster meat using seminested reverse-transcriptase polymerase chain reaction methods. Traceback investigations revealed that all oyster meat was harvested from the same estuary system in Japan within the same month.

Conclusions. These outbreaks demonstrate the potential of foodborne disease to spread internationally and the need for national and international collaboration to investigate such outbreaks. Foodborne illness related to norovirus is underestimated because of underreporting of human cases and challenges in laboratory detection of viruses in foods, both of which can delay public health action.

Noroviruses, also known as Norwalk-like viruses and human caliciviruses, are a genetically diverse group of RNA viruses of the family *Caliciviridae* [1] and are the most common causes of epidemic gastroenteritis in the developed world [2]. In the majority of outbreaks, norovirus is spread from person to person [3], but contaminated food and water are also important vehicles of transmission. The main cause of outbreaks of foodborne norovirus infection is transmission from ill food handlers, but contaminated water supplies and shellfish (in particular, oysters) have also been associated with large outbreaks [3, 4].

Oysters are filter feeders and, therefore, can concentrate viruses if they are grown in fecally contaminated waters [5]. Foodborne outbreaks of norovirus infection associated with oyster consumption have occurred in many countries [6–13] despite strategies to prevent contamination of oyster-growing areas and oyster meat [14]. The majority of outbreaks have followed the consumption of raw oysters, although cooked oysters have also been implicated [15]. The short shelf life of oysters has meant that most outbreaks have occurred locally; however, multistate outbreaks have also been reported [16].

An increase in the global consumption of seafood products has lead to an increase in the international distribution of seafood [17]. There has also been an increase in the number of outbreaks of foodborne disease associated with fish and shellfish [18], which may be related to improved surveillance but which has international implications for outbreak investigations.
and food recalls. Outbreak investigations have been assisted by the development of molecular assays for norovirus testing in foods. However, further development of laboratory capacity and sensitive assays for the detection of norovirus in oysters and other shellfish are needed [6].

We report the results of investigation into 3 outbreaks of gastroenteritis in Western Australia, Northern Territory, and Queensland between November 2003 and January 2004 associated with the consumption of raw and cooked oyster meat.

METHODS

Epidemiological Investigations

Identification of outbreaks. Gastroenteritis occurred among patrons who consumed grilled oysters that were cooked for 8–10 min at a restaurant in the Northern Territory in November 2003. At the same time, the Department of Health, Western Australia, was informed of cases of gastroenteritis among people attending a function who had consumed raw oysters used in oyster cocktails. In January 2004, several cases of gastroenteritis were reported in Queensland following consumption of raw and cooked oysters at 2 hotels. All 3 outbreaks were reported to the relevant state or territory Health Departments by either the general public or general practitioners who noticed an increased number of cases of gastroenteritis in the community. Imported oyster meat consumption was hypothesized as being the cause of all 3 outbreaks.

Human case finding. Retrospective cohort studies were conducted in the Northern Territory and Western Australia, because contact details were available for booked diners at the Northern Territory restaurant and for the attendees at the catered function in Western Australia. A case series of ill patrons with cases reported to the Health Department was conducted in Queensland. No contact details were available for other attendees at the 2 hotels in Queensland, because contact details for patrons were not recorded. A probable case was defined as occurring in a person who ate at the restaurant, function, or hotel and developed vomiting, diarrhea, or abdominal pain within 12–72 h. A confirmed case was defined as occurring in a person with a fecal specimen positive for norovirus by RT-PCR who ate at the restaurant, function, or hotel and developed vomiting, diarrhea, or abdominal pain within 12–72 h. A secondary case was defined as occurring in a person who experienced nausea, vomiting, diarrhea, or abdominal pain >72 h after a household contact had eaten at 1 of the restaurants or function centers. To confirm the start date and end date of the Northern Territory outbreak, lists of booked diners were obtained from 31 October 2003 to 3 December 2003. It was estimated that 756 people attended the restaurant during this period. Because of resource limitations, only people who ate on Friday and Saturday nights between 31 October and 19 November 2003 were interviewed. However, all diners who ate at the restaurant between 20 November and 3 December 2003 were approached for interview. In Western Australia, all patrons attending the function (87 individuals) were contacted for interview.

Subjects from the Northern Territory, Western Australia, and Queensland investigations were contacted by telephone and interviewed using a standard foodborne outbreak questionnaire. People interviewed in Northern Territory, Western Australia, and Queensland were asked whether they had eaten any of the foods served at the Northern Territory restaurant, Western Australia function, or Queensland hotels. Other questions asked included clinical history, demographic data, contact history, and whether other contacts were unwell. Data were entered into and analyzed with Stata software, version 8 (Stata Corporation). Food-specific attack rates, relative risks of illness associated with consumption of different foods at the function and restaurant, and 95% CIs were calculated. A P value of <.05 was considered to be statistically significant.

Laboratory Methods

Fecal testing. Stool samples were collected for some cases in all outbreaks. Stool samples were tested for bacteria and parasites at local state and territory laboratories and were referred to reference laboratories for viral testing, including testing for norovirus using RT-PCR methods. The reference laboratory used a hanging-drop, single-tube nested RT-PCR with appropriate controls to detect the presence of norovirus genogroup II, described elsewhere [19].

Oyster testing. Bags of oysters from the batches implicated in the outbreaks were transported frozen to laboratories in Australia and New Zealand. The New Zealand laboratory used a protease digestion method [20] to recover noroviruses from the oyster meat, followed by a seminested PCR method, which increased the sensitivity of the standard RT-PCR method.

Norovirus RT-PCR and seminested PCR. After virus recovery and viral RNA extraction from shellfish, standard norovirus RT-PCR was conducted, as described elsewhere [21]. A seminested PCR step was used to increase the sensitivity of norovirus detection in shellfish samples. Primary PCR product (1 µL) was amplified with an internal primer (Ni) [22] and the Mr9b reverse primer, using the same reaction conditions and cycling parameters for 30 cycles only. The Ni-Mr9b primer set amplifies a 114–base pair product from most genogroup I and II strains. Confirmation of norovirus presence and typing of PCR products was performed by DNA sequencing of the nested product. The seminested PCR products were purified using the QIAquick PCR purification kit (Qiagen) and were then sequenced on an ABI 3100 DNA sequencer, using Big Dye-terminator cycle sequencing methodology (Applied Biosystems). Norovirus RNA polymerase sequences from oyster and fecal samples were compared with reference sequences from

Imported Oysters and Norovirus Outbreaks • CID 2007:44 (15 April) • 1027
Table 1. Demographic and clinical characteristics from 3 outbreaks of norovirus infection associated with oyster consumption in Australia, November 2003 to January 2004.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Northern Territory</th>
<th>Western Australia</th>
<th>Queensland</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects interviewed</td>
<td>192</td>
<td>87</td>
<td>4</td>
<td>283</td>
</tr>
<tr>
<td>No. of cases associated with oyster consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probable cases</td>
<td>41</td>
<td>32</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>Confirmed cases</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>No. of cases not associated with oyster consumption</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Attack rate for oyster consumption, proportion (%)</td>
<td>45/63 (71)</td>
<td>34/43 (79)</td>
<td>...</td>
<td>79/106 (75)</td>
</tr>
<tr>
<td>Symptom frequency, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>52</td>
<td>63</td>
<td>75</td>
<td>57</td>
</tr>
<tr>
<td>Nausea</td>
<td>88</td>
<td>94</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>79</td>
<td>66</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>71</td>
<td>66</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>Fever</td>
<td>69</td>
<td>20</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>Headache</td>
<td>50</td>
<td>49</td>
<td>75</td>
<td>51</td>
</tr>
<tr>
<td>Incubation period, median h</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Duration of illness, median h</td>
<td>54</td>
<td>48</td>
<td>...</td>
<td>51</td>
</tr>
</tbody>
</table>

* A probable case was defined as occurring in a person who ate at the restaurant, function, or hotel and developed vomiting, diarrhea, or abdominal pain within 12–72 h.
* A confirmed case was defined as occurring in a person with a fecal specimen positive for norovirus by RT-PCR who ate at the restaurant, function, or hotel and developed vomiting, diarrhea, or abdominal pain within 12–72 h.
* Cases not associated with oyster consumption occurred in people who ate at the restaurant, function, or hotel and developed vomiting, diarrhea, or abdominal pain within 12–72 h but did not consume oysters.
* Attack rate was defined as the number of people who ate oysters and experienced illness divided by the total number of people who ate oysters (based on Northern Territory and Western Australia outbreaks only).

Environmental Investigation

*Site inspections and local traceback.* Interviews were conducted with the managers of the 3 food premises. Food safety staff from the Health Departments of all 3 jurisdictions contacted wholesalers about the distribution of the oyster product in their region.

*Traceback and food recall of the implicated products.* State health authorities contacted the oyster meat importers to determine international suppliers of the products with the cooperation of Food Standards Australia New Zealand and the Australian Quarantine and Inspection Service. Food Standards Australia New Zealand requested that the Ministry of Health, Labor, and Welfare in Japan obtain information about the harvest location and dates of harvest for products implicated in each outbreak.

After traceback and epidemiological evidence was obtained, the 3 importers of the oyster meat into Australia were contacted by state food safety authorities and asked to withdraw any remaining product with the same batch number.

**RESULTS**

**Epidemiological investigation.** A total of 83 people developed gastroenteritis following consumption of oysters (table 1). The attack rates in the 2 cohort studies were 71% and 79%. The median incubation period for all 3 outbreaks was 34 h (range, 12–69 h). Diarrhea was reported in 75% and vomiting in 57% of all cases. The epidemic curve shows that the Northern Territory outbreak was a continuous-source outbreak, whereas the Western Australia outbreak was a point-source outbreak (figure 1).

The duration of the Northern Territory outbreak, which was associated with grilled oysters, was 2–3 weeks. It occurred while the oysters were served at the restaurant and ceased on the day that the implicated oysters were discarded. Analysis of foodspecific attack rates and relative risks in the Northern Territory and Western Australia outbreaks showed a strong association between consumption of both cooked and raw oysters and illness. In the Northern Territory, the relative risk for illness associated with cooked oyster consumption was 17 (95% CI, 5–51; \( P < 0.01 \)). The relative risk for illness associated with oyster consumption in Western Australia was 35 (95% CI, 5–243; \( P < 0.01 \)). No other foods were associated with illness after ad-
justment for oyster consumption. In univariate analysis of the Northern Territory outbreak, consumption of chicken, noodles, or prawns was associated with illness. In Western Australia, consumption of salmon rolls was associated with illness, whereas consumption of caramel tart was negatively associated with illness. However, when consumption of oysters was considered, these associations were no longer statistically significant, because the univariate analysis was confounded by oyster consumption. Two secondary cases in the Northern Territory occurred in children or family members who did not attend the restaurant; these cases were excluded from the analysis.

Laboratory investigation. Norovirus was detected from 2 fecal specimens from cases in Western Australia, 4 cases from Northern Territory, and 2 cases from Queensland. Oysters from the Northern Territory outbreak were initially sent to 2 Australian laboratories but had test results negative for norovirus. Subsequent testing conducted in New Zealand in June 2004 detected norovirus by RT-PCR and seminested PCR methods.

Figure 2 shows the genetic relationship between norovirus reference sequences and the norovirus sequences from oysters and fecal specimens in the RNA polymerase region of the norovirus genome. Sequencing showed a range of genotypes present in the fecal specimens, including GI/4, GI/2, GII/6,7,9, GII/5, and GII/12. Three fecal specimens contained multiple norovirus strains. Sequence analysis of norovirus from 1 oyster sample showed a GII/4 strain (figure 2).

Environmental investigation. No breaches of hygiene or illnesses in staff members were identified in inspections and interviews of the staff of the 3 food premises involved in preparing the oyster cocktails and meals. Different brands of oyster meat were implicated in each outbreak, and each brand was supplied by a different Australian importer. The Ministry of Health, Labor, and Welfare in Japan indicated that the 3 different brands of oysters were supplied by a single processing company that had harvested each batch from the same harvest area in Japan over a 1-week period in February 2003. The remaining oyster meat from the same batch was voluntarily withdrawn from the market in 2 states after epidemiological and traceback evidence became available. However, a systematic withdrawal of the product across Australia did not occur until microbiological evidence of norovirus was found in the oysters, by which time most of the implicated product had been sold.

DISCUSSION

This collaborative investigation involving Australian and international authorities identified 3 norovirus outbreaks in different states and territories that were associated with consumption of the same internationally distributed batch of oysters. At the outset, our findings strongly implicated frozen oyster meat as the cause of illness, with high relative risks for illness associated with oyster consumption, a temporal relationship between consumption of oysters and illness, and consistency of this association between illness and oysters across 3 geographically distinct locations. These findings were subsequently endorsed by microbiological evidence obtained several months later. These outbreaks show the potential for international outbreaks of imported oyster meat and the need for global cooperation and food alert networks to prevent and/or limit the spread of foodborne disease.

Our investigations highlighted the debate surrounding the acceptance of epidemiological evidence in the absence of microbiological confirmation of contamination in a food product.
Conventionally, food safety agencies have based decisions regarding recall or withdrawal of a product on isolation of a pathogen from the food item. In this investigation, recall of epidemiologically implicated oysters was delayed, because norovirus was not initially detected. Consequently, a minimal amount of contaminated product was recovered from importers, and 2 Australian states and/or territories reported further cases of illness associated with consumption of oyster meat with the same batch number many months after the initial investigations (OzFoodNet, unpublished data). Since these outbreaks, collaborative effort between epidemiologists and food safety experts in Australia has resulted in discussion about the level of epidemiological evidence required for food recalls when laboratory testing for viruses in foods is unavailable.

Detection of norovirus from oyster products remains challenging despite the introduction of PCR methods, because norovirus has a variable viral genome that makes it difficult to develop a single generic detection test [23]. Human norovirus has not been grown in cell culture; therefore, standardization, inhibition of enzymes, low viral copy number, and false-negative test results can lead to difficulties in detecting norovirus in oysters [24]. Seminested PCR, which is more sensitive than standard PCR, was required to detect the norovirus from the oyster meat in this investigation. An earlier attempt (also with use of nested PCR, but conducted by a laboratory with limited experience in processing such specimens) was unsuccessful. This highlights the need for laboratories to regularly process samples to ensure that testing quality and sensitivity is achieved and maintained. PCR-based surveys of oysters in Asia have shown that up to 10% of oyster meat may be contaminated with multiple strains of norovirus [25]. Using more-sensitive PCR methods, some researchers have shown higher rates of oyster contamination [26].

Multiple strains of norovirus were detected from fecal specimens and from 1 batch of oyster meat. The isolation of multiple norovirus strains has previously been reported in association with outbreaks of norovirus infection implicating shellfish [5, 23, 27–29]. Kageyama et al. [27] sequenced norovirus from 66 gastroenteritis outbreaks in Japan and found that all shellfish-related outbreaks were associated with multiple genotypes. Oysters are filter feeders and concentrate enteric viruses when grown in fecally contaminated water. In our investigation, the source of fecal contamination in the harvest area was never identified, but a previous study [30], which was conducted in Japan, showed that oysters intended for raw consumption and harvested in the months of January or February were highly contaminated with diverse strains of norovirus. The contamination of oysters in Japan is an ongoing problem, with surveillance data over a 34-month period showing that 154 (54%) of 287 foodborne norovirus outbreaks were related to oyster consumption [31].

To safeguard consumers, oyster producers have made efforts to reduce norovirus infection following oyster consumption through the use of water testing at the harvest site, washing oyster meat, and printing cooking advice on packaging. None of these measures were effective in preventing these outbreaks in Australia. Inactivation of norovirus varies according to the method of cooking and the internal temperature of the oyster. Norovirus is heat resistant [32], with viable virus surviving after heating at 60°C for 30 min [33]. In other outbreak investigations, people eating well-cooked or over-cooked shellfish had a risk of becoming ill that was similar to that for people who ate raw shellfish [5, 15]. It is likely that the time and temperature of cooking required to inactivate norovirus in oysters may render the food unpalatable to consumers [32, 34]. Restaurants and the general public in many countries assume that oysters are safe to consume raw, and Australia and New Zealand have experienced several outbreaks linked to similar frozen oyster meat products in which warning labels on packages stating that the product should be cooked before consumption were ignored (OzFoodNet, unpublished data; Institute of Environmental Science and Research, unpublished data).

Contamination of oyster beds with sewage effluent from oyster harvesting boats and recreational boats has been reported in the United States and New Zealand [13, 16, 35]. In many countries, oyster beds are located in bays and estuaries close to residential areas [23, 28]. It is difficult for countries importing oysters to ensure that oysters are sourced from areas where fecal contamination cannot occur. As a result of our investigation, the Australian Quarantine and Inspection Service restricted importation of oysters from the implicated estuary system.

International collaboration between Australian and Japanese authorities was essential for traceback of the implicated product. The investigations highlight the usefulness of establishing and maintaining information-sharing networks globally to allow for the rapid exchange of information between food regulatory agencies across the world in times of food safety emergencies. Information sharing provides countries with intelligence on emerging infections and assists with the development of control strategies to prevent disease [36]. The World Health Organization recently developed a new electronic International Food Safety Authorities Network to allow for the sharing of information on food safety and contamination incidents [37]. The International Food Safety Authorities Network may be a useful tool in the future to assist countries to disseminate information about products that cause outbreaks of foodborne disease. The international public health community needs to continue to improve these networks and to develop new ones to deal with a global food supply and international foodborne outbreaks.
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