Outbreak of Norwalk virus in a Caribbean island resort: application of molecular diagnostics to ascertain the vehicle of infection

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(Accepted 1 January 2001)

SUMMARY

In 1998, an outbreak of gastroenteritis affected at least 448 persons including 122 staff at a resort hotel in Bermuda. A survey among staff indicated that gastroenteritis was associated with eating or drinking at the hotel (OR = 6.0, 95% CI = 2.4–15.1). Multiple specimens of drinking water had elevated faecal coliform levels and Escherichia coli present, suggestive of faecal contamination. Stools from 18 of the 19 persons with gastroenteritis that were tested were positive for genogroup-II Norwalk-like viruses (NLVs). RT–PCR analysis of a 3 l specimen of water produced a genogroup-II NLV genome with a sequence identical to that of NLVs in the stools of three ill persons. This outbreak shows the value of new molecular diagnostics to link illness with a contaminated source through the use of sequence analysis. The risk of outbreaks such as these could be reduced in tourism dependent regions like Bermuda and the Caribbean by regular evaluation of data from the inspection and monitoring of drinking water supplies and waste water systems, by ensuring the chlorination of supplemental drinking water supplies and by establishing food-safety initiatives.

INTRODUCTION

The ‘Norwalk-like viruses’ (NLVs), also called small round-structured viruses, are a major cause of outbreaks of gastroenteritis worldwide [1]. Food and waterborne NLV outbreaks are common and have been linked to exposure to contaminated vehicles of infection such as salads, celery, shellfish (e.g. raw oysters, clams), sandwiches, swimming water, drinking water and ice [2–13]. Because NLVs cannot be propagated in cell culture or animal models, diagnostic methods have relied on the examination of faecal specimens to visualize viral particles by electron microscopy (EM), detection of the viral genome by using reverse transcriptase-polymerase chain reaction (RT–PCR), and measurement of antibody responses in infected individuals [14, 15]. These techniques have provided a sensitive and specific means to confirm the aetiology of NLV outbreaks and to identify persons with recent infection. However, the sensitivity to detect small numbers of NLVs in vehicles epidemiologically implicated in food and water outbreaks is low, and RT–PCR has not yet been widely used for this purpose. We investigated an outbreak of gastroenteritis among persons at a resort in Bermuda and...
applied molecular assays to identify NLVs in both faecal specimens and the implicated vehicle of transmission.

BACKGROUND

Bermuda is a constellation of small islands in the Atlantic Ocean about 568 miles east of the United States of America with a land area of 22 square miles and no natural water sources. The residents depend on rainwater collection for water supply, which is collected off limestone-coated roofs and channeled into underground storage tanks. Households tend to use this water untreated while institutions may chlorinate it. While the Bermuda Department of Health (BDOH) recommended chlorination of rainwater catchments, there was no legal requirement to do so. Storage tanks are required to be emptied, scrubbed and sanitized every 6 years. The government also supplies treated water drawn from rainwater catchments and wells, which is piped or occasionally trucked to specific institutions. Institutions such as hotels often obtain their water from a variety of sources, including the government mains, rainwater catchments, desalination of seawater, or a private water treatment company.

On 10 February 1998, the BDOH was notified of gastrointestinal illness among 14 foreign guests of a large resort hotel with 402 rooms and a capacity of 900 persons. Five days later additional persons with illness were reported with some visiting the local emergency room after attending various functions at the hotel. On 21 February, the hotel was closed because of the numbers of reported cases and the presence of faecal coliforms in the potable water supply. On 23 February, the BDOH invited a team from the Caribbean Epidemiology Centre (CAREC) to assist with an investigation to identify the causative agent and its mode of spread, and to help with control and prevention measures.

METHODS

Epidemiological studies

A suspected case of gastroenteritis was defined as a foreign guest, local patron, or worker at the hotel who developed either nausea, vomiting, diarrhoea (3 or more loose stools in a 24 h period), or abdominal cramps during 5–21 February 1998. To identify cases, we interviewed the hotel staff, and patients identified from the telephone hot-line reports, the local hospital’s emergency room log, the hotel physician’s log, the hotel’s complaints log and listings of ill persons reported by organizers of functions held at the hotel. Attack rates of illness were calculated by using denominators estimated from daily hotel occupancy levels, the staff complement for the period of the study, and the hotel’s estimates of the sizes of specific groups that had visited during the period. Because some staff were contracted on an as-needed basis, the hotel’s management was unable to provide the exact staffing numbers by department during the outbreak. Department-specific attack rates were calculated by assuming a full staff complement and thus the actual rates may be higher than those reported. Likewise, because some guests may not have reported their illness, we may have underestimated the attack rate among guests.

To identify exposures associated with gastroenteritis, a survey was administered to the hotel staff. Since the hotel had discharged most of the foreign guests or they had left before the investigation began, we attempted to survey guests and patrons that attended various functions, but the response rate was too low to provide meaningful results. The associations between illness and various exposures among hotel staff respondents were ascertained by calculating the maximum likelihood estimate (MLE) of the odds ratio (OR) and exact 95% confidence limits (CIs) for the MLE. Analyses were performed by using Epi Info, Version 6.04 software [16].

Environmental studies

Environmental investigations focused on food items served at the hotel and its water supply. Foods eaten at the hotel were identified from the menu for various groups, functions and restaurants during 5–21 February 1998. However, no food items were available for microbiologic analysis.

Water was collected from the terrace tank and at various sites along the hotel’s potable water supply and tested for coliform bacteria and residual chlorine. Three litres of water collected from the terrace tank was sent to the Centers for Disease Control and Prevention (CDC), Atlanta to test for viral organisms. The potable and waste water systems of the hotel were reviewed to identify opportunities for contamination of the potable water system and deficiencies in the management of these systems. The hotel maintained no logs or procedural guidelines for its water supply so we constructed a profile of the existing systems.
Microbiological studies

Stool specimens from 35 ill persons were screened locally for bacteria and parasites. Of these 23, were also tested for bacteria and parasites at CAREC. Specimens were sent for additional laboratory testing: 19 were sent to CDC for vital detection, of which 6 were also sent to the BBI–North American Clinical Laboratories in Boston, MA for bacterial and viral testing.

The BBI laboratory tested for vibrio, entero haemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), listeria, S. aureus, giardia (microscopic and EIA), cryptosporidium (microscopic and EIA), cyclospora (microscopic) and viruses including adenovirus, enteric viruses and rotavirus. The CAREC lab tested for salmonella, shigella and campylobacter. At CDC, faecal specimens were tested at the Foodborne and Diarrheal Diseases Branch for ETEC and at the virology laboratory for NLVs. Virologic testing at CDC included RT–PCR and the sequencing of amplicons from selected samples using previously described methods [15]. Several weeks later and in a manner to ensure no possible cross-contamination, the 3 l water sample was filtered through electro-positive filters for virus adsorption (Zetapor® Membrean, Cuno, Inc., Meridian, CT). The virus was eluted from the filters using the methods described by Beller et al. [10], with slight modification. The final virus pellets were resuspended in sterile water and RNA was extracted and amplified using the G1 and G2 primer sets described by Ando et al. [15]. The nucleotide sequence of the resulting PCR amplicons was compared with those from the stool samples and with the available sequences in the public databases.

RESULTS

Epidemiological findings

Our investigation identified 448 persons (142 in-house foreign guests, 122 hotel-staff, and 184 local patrons who used the hotel facilities) with at least one gastrointestinal symptom. The most commonly reported symptom was diarrhoea (n = 290, 65%), followed by nausea (n = 276, 62%), vomiting (n = 262, 58%) and abdominal cramps (n = 178, 40%). Of the 448 persons, 58 (13%) presented with all 4 symptoms, 127 (28%) had 3 symptoms, 130 (29%) had 2 symptoms, and 133 (30%) had 1 symptom. The incubation period could be most reliably estimated for the local patrons who visited the hotel on a specific day, 101 of whom visited on 14 February, Valentines Day. Of the 184 patrons, the date of visit to the hotel and date of onset of symptoms was available for 156, of whom 94 (60%) had an incubation period of 1–2 days.

Based on the dates of onset for 401 suspected cases, the outbreak began on 7 February and peaked on 15 February (Fig. 1). The 17 confirmed cases showed a similar pattern (Fig. 2). During the period 7–21 February, the estimated minimum cumulative attack rate for foreign guests was 16% (142/868) and, for subgroups of foreign guests for whom accurate estimates could be made, attack rates were much greater. For example, the cumulative attack rate for a group of 109 golfers was 40% and for a group of 79 from a pharmaceutical company it was 54%. The hotel had a roster of 462 staff (289 full-time, 74 part-time, 99 temporary) and approximately 375 were employed during February. The highest attack rates for staff, 19% (31/163) and 16% (29/180), were recorded on
Fig. 2. Epidemic curve of confirmed suspect cases of gastrointestinal illness among guests, staff, and patrons, Bermuda hotel, February 1998. HS, hotel staff, persons who worked at the hotel; HP, hotel patron, persons who went to restaurants or functions at the hotel; HG, hotel guest, persons who stayed at the hotel.

Table 1. Risk factors associated with illness among staff, Bermuda Hotel, February 1998 (n = 184)

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Number of ill staff</th>
<th>Number not ill</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate/dranked at hotel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>104</td>
<td>33</td>
<td>6.0</td>
<td>2.4–15.1</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ill family member</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28</td>
<td>4</td>
<td>6.4</td>
<td>1.0–12.5</td>
</tr>
<tr>
<td>No</td>
<td>84</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live in staff dormitory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>100</td>
<td>20</td>
<td>0.16</td>
<td>0.01–0.52</td>
</tr>
</tbody>
</table>

Denominators differ because of non-response to items.

12 and 13 February respectively, and the estimated cumulative attack rate was 33% (122/375).

We assessed exposures associated with illness by surveying hotel staff. Of the 184 respondents, 122 (66%) reported gastroenteritis. Those who ate and/or drank at the hotel were significantly more likely to report gastroenteritis than those who did not (OR = 6.0, CI = 2.4–15.1) (Table 1). Having an ill family member was also associated with illness (OR = 3.4, CI = 1.0–12.5). The risk associated with eating and/or drinking at the hotel remained after adjusting for having an ill family member (Mantel–Haenszel adjusted OR = 5.8, CI = 2.4–13.4). The survey did not assess specific food and water items.

Environmental findings

The resort’s water was supplied from rainwater catchments and the treated Government supply and stored in a subterranean concrete terrace tank with a capacity of 600 000 gallons located adjacent to the hotel in front of restaurant A (Fig. 3). This tank apparently also received other water from sources that
Table 2. Water quality reports, Bermuda hotel, February 1998

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Date collected</th>
<th>Date reported</th>
<th>Coliforms (/100 ml)</th>
<th>Faecal coliforms (/100 ml)</th>
<th>E. coli (/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banquet kitchen</td>
<td>10 Feb 98</td>
<td>13 Feb 98</td>
<td>CG</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>Fifth floor ice machine</td>
<td>16 Feb 98</td>
<td>18 Feb 98</td>
<td>300</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Golf grill kitchen ice machine</td>
<td>16 Feb 98</td>
<td>18 Feb 98</td>
<td>CG</td>
<td>≥ 189</td>
<td>≥ 116</td>
</tr>
<tr>
<td>Main kitchen ice machine</td>
<td>16 Feb 98</td>
<td>18 Feb 98</td>
<td>CG</td>
<td>≥ 126</td>
<td>84</td>
</tr>
<tr>
<td>Main kitchen banquet sink</td>
<td>16 Feb 98</td>
<td>18 Feb 98</td>
<td>CG</td>
<td>≥ 490</td>
<td>≥ 400</td>
</tr>
<tr>
<td>Government supply pre terrace tank</td>
<td>16 Feb 98</td>
<td>18 Feb 98</td>
<td>&lt; 4</td>
<td>&lt; 1</td>
<td>—</td>
</tr>
<tr>
<td>Terrace tank</td>
<td>16 Feb 98</td>
<td>18 Feb 98</td>
<td>CG</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>Terrace tank while being emptied</td>
<td>16 Feb 98</td>
<td>18 Feb 98</td>
<td>CG</td>
<td>95</td>
<td>46</td>
</tr>
<tr>
<td>Cast iron pipe inside tank</td>
<td>19 Feb 98</td>
<td>23 Feb 98</td>
<td>140</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>Opening in tanks south-east corner</td>
<td>19 Feb 98</td>
<td>23 Feb 98</td>
<td>286</td>
<td>79</td>
<td>60</td>
</tr>
</tbody>
</table>

All samples taken after 20 Feb 1998, had coliform and faecal coliform counts < 1.

CG, Confluent growth: the colonies had overgrown and merged and could not be identified individually.

<table>
<thead>
<tr>
<th>Outbreak 12C/92/UNK</th>
<th>1</th>
<th>CTCTCTACCCCTATAGGCGGCTTTCTGAATTTAGACGACTCTCTCTGACAT</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outbreak 12C/92/UNK</td>
<td>282</td>
<td>CTCTCTACCCCTATAGGCGGCTTTCTGAATTTAGACGACTCTCTCTGACAT</td>
<td>331</td>
</tr>
<tr>
<td>Outbreak 12C/92/UNK</td>
<td>51</td>
<td>TGTCAGGCGCCACTGCCCTCTCTCTGTTTTAT</td>
<td>81</td>
</tr>
<tr>
<td>Outbreak 12C/92/UNK</td>
<td>332</td>
<td>TGTCAGGCGCCACTGCCCTCTCTCTGTTTTAT</td>
<td>362</td>
</tr>
</tbody>
</table>

Fig. 4. Alignment of the 81-base unique sequence of the RT-PCR products from stool and water samples collected during the outbreak investigation with the corresponding region of human calicivirus strain 12C/92/UNK (GenBank L25111).

could not be identified. Rainwater was routed from its point of origin into underground tributaries and flowed into open channels, which emptied into the terrace tank. This rainwater was then pumped into an elevated 50 000-gallon holding tank from which it flowed by gravity throughout the hotel.

Sanitary inspection of the water supply identified a number of deficiencies. The access cover for the terrace tank was rusted, improperly secured and sunken below ground level, allowing surface run-off water to enter the tank. The tank had been last cleaned more than 5 years before and debris was found at the bottom of the access to the tank. The water was not chlorinated. After excavation, a large trapped collection of water along with an open Y-shaped cast iron pipe was observed under the edge of the hotel adjacent to the lobby of restaurant A. Dye flushed down the cast iron pipe flowed into the underground terrace tank indicating that if overflow water from the toilets and the wastewater channels near restaurant A entered this body of water, it would have entered the tank. Local patrons reported that prior to 14 February, many of the bathrooms were out of service and the area near restaurant A was flooded and had a detectable odour of faeces.

Portions of the underground sewage system and rainwater channels ran parallel but counter-current to each other. Access to both systems was possible through separate manholes near the main kitchen. A concrete wall, approximately 6 inches thick, separated the two systems, but a large piece of the separating wall between the manholes had broken off, obstructing the free flow of sewage and wastewater and consequently caused sewage to overflow into the rainwater channel via the manhole access. Microbiological analyses of water samples collected between 16 and 19 February at various points along the hotel’s distribution system and from the terrace tank showed widespread faecal contamination within the hotel (Table 2). Only the sample taken on 16 February from the government supply, at a point prior to its entry into the terrace tank, was free of contamination.

Laboratory findings

Eighteen persons tested positive for NLV, 10 were foreign guests who had stayed at the hotel, 5 were members of staff and 3 were local patrons who had visited the hotel. No bacterial or parasitic pathogens were identified in the faecal specimens. NLVs were
identified by EM in 9 of 13 stools examined and by RT–PCR using genogroup II primers in 18 of 22 stools tested. In total, specimens from 18 of the 19 ill persons were positive for NLVs by either EM alone (4 persons), RT–PCR alone (5 persons), or both EM and RT–PCR (9 persons). The water specimen was positive for NLVs using the genogroup II primers. The nucleotide sequence of the amplicon from the water was identical to those determined from the three stool specimens for which sequencing was performed. This sequence was 96.3% identical to the sequence of strain 12C/92/UNK [17] over the 81-base region of overlap (Fig. 4).

DISCUSSION

The results of this investigation demonstrated that ingestion of faecally contaminated drinking water and/or ice played an important role in amplifying this large outbreak of NLV gastroenteritis. The clinical presentation of the case-patients and the incubation period was consistent with infection by NLVs [18]. The high attack rate among foreign guests and staff was consistent with waterborne spread and the staff survey suggested a link between illness and eating or drinking at the hotel. This link was confirmed by the demonstration of faecal contamination of samples from the hotel's water supply and by the detection of the NLV genome that shared a common sequence from ill persons and the water sample collected from the terrace tank. Our investigation identified at least two potential modes of water contamination. A blockage in the underground sewage system may have resulted in a backup of sewage and wastewater at a number of locations near the opening of the terrace tank and could have resulted in the contamination of inflowing water. Intermittent seepage of sewage into the rainwater system through a breach in the separating wall might also have contributed to the contamination. The fact that only one genome was detected suggests that one patient may have been the source of the organism. However, our investigation could not distinguish between the possibility that the original source may have been hotel guest(s) with foodborne illness, or an employee with community-acquired illness.

The prospect of detecting viral genome in relatively small amounts of water offers an exciting new opportunity for more thorough investigations of NLV waterborne outbreaks. Beller et al. were the first to use nested PCR testing of concentrates of water from a contaminated well to confirm that a NLV outbreak affecting tour groups in Alaska was associated with consumption of water from the well [10]. Multiple bulk water samples (7.5–9.0 l per sample) from the well were filtered to yield the concentrates that were tested. Kukkula et al. recently reported the detection of NLV genome in concentrates obtained by filtration of multiple 1 l water specimens obtained during the investigation of a waterborne NLV outbreak in Finland [11]. In this outbreak, we detected NLV genome from the single 3 l bulk sample of contaminated water that was collected during the investigation, which suggests that the water in this outbreak must have been very contaminated.

As a control measure, the terrace-tank was taken offline and the government water supply routed to the holding-tank. On 23 February, water from the holding-tank was super-heated and hyper-chlorinated and used to flush the water system throughout the hotel. Residual chlorine levels were checked at distal points of the hotel’s water supply to ensure that adequate levels of chlorine were maintained for an appropriate contact time. The hotel’s water supply was monitored daily for residual chlorine and faecal coliforms, and samples were collected periodically for bacterial and viral testing. All samples taken after 20 February had coliform and faecal coliform counts < 1. Based on these results, the hotel was reopened to in-house foreign guests only on a limited basis on 26 February, using one wing, one restaurant and one kitchen. All foreign guests were informed about the problem and the investigation, signs were posted in the bathrooms, bottled water was provided, and an active surveillance system was set up to identify foreign guests or staff with illness.

This investigation underscores the need to reassess the safety of drinking water supplies in regions of the world where the pre-existing systems may be outdated and prone to malfunction or contamination. Information about the potable and waste water systems at the hotel where this outbreak occurred was incomplete because of the absence of appropriate engineering records and maintenance logs and the lack of institutional memory due to frequent changes in management. The risk of outbreaks could be reduced by ensuring the regular evaluation of data from the inspection and monitoring of drinking water supplies and waste water systems, and by ensuring the chlorination of supplemental drinking water supplies. Compliance with the recommendation to clean subterranean water tanks every 6 years was not known.
but this recommendation should vary based upon the size and age of the tank, and the number of people potentially at risk. Despite the fact that NLVs are a common cause of acute gastro-intestinal outbreaks [1] this was the first documented occurrence of a NLV outbreak reported to CAREC. Despite the availability of new diagnostics these techniques are not widely used in areas such as the CAREC member countries, and other countries of the Caribbean and Latin America where they could be of use. Given the rapid and extensive development of tourism in areas like the Caribbean, countries should consider establishing food- and water-safety initiatives to regularly inspect and monitor drinking water supplies and waste water systems in the food service industry, including active surveillance for the early detection and control of outbreaks. Caribbean Ministries of Health should sensitize the public and foodservice industries to methods, such as the Hazard Analysis and Critical Control Point program [19] and hotel (guest and staff)-based surveillance systems being implemented by CAREC and some Governments [20]. Standard existing technologies such as screening for coliforms in water would be enhanced by the use of modern laboratory technologies to support investigations and the periodic monitoring of water supplies.

ACKNOWLEDGEMENTS

We acknowledge the following persons and agencies for their assistance and technical advice during this investigation: Rhonda Daniels, Nurse Epidemiologist, Estlyn Harvey, Chief Environmental Health Officer and other staff of the Bermuda Department of Health; Sue Smith, Head Microbiologist, and other staff of the King Edward VII Hospital Laboratory, Bermuda; Stephen S. Monroe, PhD, Charles D. Humphrey, PhD, and Roger I. Glass, MD, PhD, Viral Gastroenteritis Section, Division of Viral and Rickettsial Diseases, National Center for Infectious Disease, Centers for Disease Control and Prevention, Atlanta, GA; Diarrheal Diseases Laboratory Section, Foodborne and Diarrheal Diseases Branch, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA; University of North Carolina at Chapel Hill, NC, USA; BBI–North American Clinical Laboratories, CT, USA; the management and engineering staff of the resort in Bermuda; the laboratory staff at the Caribbean Epidemiology Centre, Port of Spain, Trinidad & Tobago.

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