Outbreak of Viral Gastroenteritis Due to a Contaminated Well

International Consequences

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Context.—Small round-structured viruses (SRSVs) are known to cause viral gastroenteritis, but until now have not been confirmed in the implicated vehicle in outbreaks.

Objective.—Investigation of a gastroenteritis outbreak.

Design.—After applying epidemiologic methods to locate the outbreak source, we conducted environmental and laboratory investigations to elucidate the cause.

Setting.—Tourists traveling by bus through Alaska and the Yukon Territory of Canada.

Participants.—Staff of a restaurant at a business complex implicated as the outbreak source, convenience sample of persons on buses that had stopped there, and bus employees.

Main Outcome Measures.—Odds ratios (ORs) for illness associated with exposures. Water samples from the restaurant and stool specimens from tourists and restaurant staff were examined by nucleic acid amplification using reverse transcription polymerase chain reaction and sequencing of viral amplification products.

Results.—The itineraries of groups of tourists manifesting vomiting or diarrhea were traced back to a restaurant where buses had stopped 33 to 36 hours previously. Water consumption was associated with illness (OR, 5.3: 95% confidence interval [CI], 2.3-12.6). Eighteen of 26 employees of the business complex were ill; although not the index case, an employee ill shortly before the outbreak lived in a building connected to a septic pit, which was found to contaminate the well supplying the restaurant’s water. Genotype 2/P2B SRSV was identified in stool specimens of 2 tourists and 1 restaurant employee. Stools and water samples yielded identical amplification product sequences.

Conclusions.—The investigation documented SRSVs in a vehicle epidemiologically linked to a gastroenteritis outbreak. The findings demonstrate the power of molecular detection and identification and underscore the importance of fundamental public health practices such as restaurant inspection, assurance of a safe water supply, and disease surveillance.

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SMALL ROUND-STRUCTURED VIRUSES (SRSVs), also called Norwalk-like viruses, are human enteric viruses in the family Caliciviridae. These viruses have not been cultivated in vitro, do not have a practical animal model in which they can be propagated, and are shed in relatively low numbers for only a short time during human illness. Nevertheless, SRSVs have been established as the major cause of viral gastroenteritis among adults worldwide. Outbreaks due to person-to-person or common-source transmission by food handlers, and consumption of contaminated oysters, ice, and celery have occurred. Waterborne outbreaks involving community water systems and contaminated wells have been reported. Transmission via aerosolized vomitus may be possible. Although epidemiologic and laboratory investigations of SRSV outbreaks have been thorough, none has identified SRSVs in the incriminated vehicle. We investigated a gastroenteritis outbreak in tourists traveling by tour bus between the United States and Canada, which provided an opportunity to develop novel laboratory methods not previously used to study SRSVs.

METHODS

Background

The outbreak was reported to the Alaska Division of Public Health after a Fairbanks, Alaska, hotel operator recognized that ill bus passengers were staying at the hotel. Ill bus passengers were also identified in hotels in Skagway and Valdez, Alaska; the investigation subsequently implicated a restaurant in the Yukon Territory of Canada in a small community on the Alaska Highway. The restaurant, which catered largely to
summer tourists traveling by bus, was part of a complex including a motel.

At least 8 tour bus companies made lunch stops at the restaurant; company "A" carried nearly 80% of passengers. This company stopped there on 6 partially overlapping itineraries: Skagway to Fairbanks; Skagway to Valdez; Haines, Alaska, to Beaver Creek, Yukon Territory; and, for each, the reverse direction of travel. Each trip lasted 3 days (except between Haines and Beaver Creek, which was a 1-day trip) and included up to 9 meals at the same restaurants and hotels along each segment. During the outbreak, company A made 59 stops at the implicated restaurant, while 5 other companies made 7 stops. Company A ceased using the implicated restaurant after June 29. However, cases identified from other companies after that date were included in the analysis.

Epidemiologic Investigation

Data concerning illness on 10 such bus trips during June 25 through July 11, 1995, were collected using methods that depended on accessibility of passengers (Table 1). Initially we interviewed ill persons and travel companion(s) on trips 1 and 3 at a Fairbanks hotel. We further studied trip 1 by systematically sampling 50 persons for interview while traveling by train from Fairbanks to Denali National Park. Each person was questioned about demographics, symptoms, and food and water consumption during the period beginning 48 to 72 hours prior to illness onset. Attack rates for trips 3, 4, 5, and 6 were estimated via telephone interview of persons about 56 hours after eating at the restaurant. A case was defined as a person having vomiting or diarrhea (3 or more loose stools in any 24-hour period) after traveling by bus on any part of the Alaska Highway between Whitehorse and Beaver Creek during June or July. We relied on information provided by hotel staff or company A employees to characterize illness on trips 2, 7, and 8.

The probable source of the outbreak was identified by determining if there were restaurants or hotels for which time intervals between meals and illness onset were about equal for each itinerary, based on the hypothesis that (cause and source) was the same for ill persons in Fairbanks, Skagway, and Valdez, and that illness onset would occur after about the same time interval following a common-source exposure for each trip. Mean time of illness onset was determined via interview of persons on trips 1, 2, 3, 9 (data were obtained from only 12 persons on trip 3), 8, and 10. Persons on trips 4, 5, and 6 were not asked the exact time of becoming ill; illness onsets were considered the same as either other persons on the same itinerary (for trips 4 and 6) or persons on trips 1, 2, and 3 (for trip 5, based on hypothesized common source and time of exposure). Meal times, rounded to nearest hour, were obtained from bus companies.

Restaurant employees were interviewed by local public health personnel, and we surveyed 30 of 46 company A employees who had stopped there in June. Employees were asked about symptoms, items consumed at the restaurant, and when bus passengers were first noted to be ill.

Environmental Investigation

We inspected the restaurant on July 12, 13, 18, and 19. Fluorescein dye was flushed down a toilet in the motel, and water samples were collected for coliform bacteria, fluorescein, and viral testing.

Laboratory Investigation

Stool specimens were collected from 9 ill passengers on trips 1 and 3 and tested for Salmonella, Shigella, Campylobacter, and Yersinia. Fourteen other ill passengers later provided 17 stool specimens (9 were primary cases, the other were secondary cases), which were forwarded to the US Centers for Disease Control and Prevention (CDC) for electron microscopy and molecular diagnostics. Molecular methods comprised reverse transcription polymerase chain reaction (RT-PCR) for amplification of a 123-base pair portion of the SRSV RNA polymerase gene, Southern hybridization for identification of amplification products, and nucleotide sequencing.\textsuperscript{31,32}

Local public health officials collected stools from 5 ill and 6 well employees of the implicated business complex (no stools were collected from bus employees). Routine cultures for bacterial pathogens were negative. Two of the 5 ill employees had onset of symptoms 6 days or less before specimen collection and we arranged for their 2 stools to be forwarded to the Hospital for Sick Children (Toronto, Ontario) for SRSV-specific RT-PCR using a procedure similar to that followed by CDC.\textsuperscript{33} The RT-PCR products were genotyped by blot hybridization and then, after the National Centre for Enteroviruses (Halifax, Nova Scotia) had completed the water concentrate analyses, sent there for sequencing.

Bulk water samples (7.5 to 9.0 L per sample) from the implicated business complex were processed for virus concentration and purification at the University of North Carolina, Chapel Hill, using a novel 2-step procedure modified from previous methods.\textsuperscript{34,36} Samples were filtered through double layers of

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Table 1.—Gastrointestinal Illness In Tour Bus Travelers, Yukon Territory and Alaska, June and July 1995

<table>
<thead>
<tr>
<th>Trip No.*</th>
<th>Date at Implicated Restaurant</th>
<th>Itinerary</th>
<th>Method of Investigation</th>
<th>Onboard</th>
<th>Interviewed†</th>
<th>Ill</th>
<th>Attack Rate‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/25</td>
<td>Skagway to Fairbanks</td>
<td>Hotel, face-to-face interviews</td>
<td>135</td>
<td>9</td>
<td>7</td>
<td>. . .</td>
</tr>
<tr>
<td>2</td>
<td>6/25</td>
<td>Skagway to Valdez</td>
<td>Tour bus company interviews</td>
<td>41</td>
<td>0</td>
<td>10</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>6/26</td>
<td>Skagway to Fairbanks</td>
<td>Hotel, face-to-face interviews</td>
<td>121</td>
<td>12</td>
<td>6</td>
<td>. . .</td>
</tr>
<tr>
<td>4</td>
<td>6/27</td>
<td>Skagway to Valdez</td>
<td>Telephone survey</td>
<td>. . .</td>
<td>50</td>
<td>9</td>
<td>0.18</td>
</tr>
<tr>
<td>5</td>
<td>6/27</td>
<td>Fairbanks to Skagway</td>
<td>Telephone survey</td>
<td>. . .</td>
<td>50</td>
<td>9</td>
<td>0.18</td>
</tr>
<tr>
<td>6</td>
<td>6/27</td>
<td>Skagway to Fairbanks</td>
<td>Telephone survey</td>
<td>. . .</td>
<td>50</td>
<td>9</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>6/29</td>
<td>Fairbanks to Skagway</td>
<td>Reported by hotel staff</td>
<td>. . .</td>
<td>25</td>
<td>0</td>
<td>. . .</td>
</tr>
<tr>
<td>8</td>
<td>6/29</td>
<td>Skagway to Fairbanks</td>
<td>Reported by hotel staff</td>
<td>. . .</td>
<td>25</td>
<td>0</td>
<td>. . .</td>
</tr>
<tr>
<td>9</td>
<td>7/9</td>
<td>Whitehorse to Fairbanks</td>
<td>Face-to-face interviews</td>
<td>. . .</td>
<td>28</td>
<td>17</td>
<td>0.61</td>
</tr>
<tr>
<td>10</td>
<td>7/11</td>
<td>Valdez to Skagway</td>
<td>Face-to-face interviews</td>
<td>. . .</td>
<td>29</td>
<td>5</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td>654</td>
<td>274</td>
<td>108</td>
<td>. . .</td>
</tr>
</tbody>
</table>

*Trips 1-8 were operated by tour bus company A. Trips 9 and 10 were operated by other companies.
†Interviewed by 1 or more of the authors.
‡Attack rates were not determined when ill persons were specifically targeted to be interviewed (trips 1 and 3, hotel) or if they were reported by hotel staff but their entire group was not studied (trips 7 and 8).
§Two persons on trip 3 were included in both the hotel interviews and the telephone survey.
| One person was counted twice due to being interviewed twice. |
RESULTS

Epidemiologic Investigation

Overall, of the 654 bus passengers represented in the trips analyzed in this report, 274 persons were interviewed (there were no refusals) and 108 met the case definition. Detailed data on symptoms were collected from 54 ill passengers and 10 ill bus company A employees; most frequent symptoms were diarrhea, vomiting, and nausea (Table 2). Since most ill persons were symptomatic when interviewed, mean duration of illness was not calculated; we observed that many were improving within 24 to 48 hours. A minimum of 6 persons required hospitalization for dehydration or correction of electrolyte abnormalities. Onset dates for passengers ranged from June 26 to July 12 (Figure 1). Age was recorded for 100 of 128 passengers interviewed face to face; median age of 42 cases, 65.0 years, was similar to that of 58 persons not meeting the case definition, 64.5 years. We did not investigate reports of secondary illness.

After interviewing passengers on the first 6 trips, but before the etiologic agent was identified, we evaluated meal to onset time intervals (Table 3). This analysis showed that the interval between lunch at the restaurant and mean time of illness onset was 33 to 36 hours for each itinerary. Time intervals between meals at other locations and mean illness onset were unequal. Assumptions used to calculate illness onset times for trips 4, 5, and 6 appeared to be valid since passengers on these trips had onset about 32 hours after eating at the restaurant.

We asked passengers on trips 4, 5, 6, 9, and 10 about items consumed at the restaurant. There was an association between water and illness (odds ratio [OR] = 5.3, 95% confidence interval [CI], 2.3-12.6); no foods were associated with illness (Table 4). We collected more detailed information about water consumption from persons on trip 10. Although results were possibly consistent with a dose-response relationship (attack rates were 0% [0/15] in persons who did not drink water vs 33% [1/3], 38% [8/3], and 33% [1/3] in those who drank <1 L, 1 L, and >1 glass, respectively), the trend was not statistically significant. The person considered the sickest on this trip (vomiting more than 20 times) also had the greatest consumption of water (2-3 glasses).

All but 3 of 26 business complex employees lived on-site and all 26 were interviewed (24 by local public health personnel and 11 by us); 18 had acute gastroenteritis during June or July (attack rate = 69%). Ten employees recalled exact day of onset; 1 living in the motel had diarrhea beginning June 13 (Figure 1). Three nonemployees also living in the motel had diarrhea beginning June 14.

Ten company A employees reported vomiting or diarrhea (attack rate = 33%); onset ranged from June 21 to July 1 (Figure 1). Symptoms were similar to those of passengers, although a larger proportion reported fever (Table 2). Water consumption and illness were associated (9 of 16 employees who drank water and 1 of 11 who did not were ill [3 employees could not recall if they drank water]; OR = 12.9, 95% CI, 1.2-618.5). Ill passengers were noticed by 18 employees; earliest onset date, June 21, related to persons who had eaten at the restaurant on June 20 (not included in our analysis).

Attack rates by trip varied from 17% to 61% (Table 1), with a weighted mean of 25%. Although we investigated only 10 of 46 trips that stopped at the restaurant between June 20 and July 11, we were notified of illness on most trips. Investigated trips were chosen for convenience and were believed to be similar to the others. By applying the mean attack rate to the reported 1732 persons on the 46 trips, 433 primary cases were estimated. If highest and lowest attack rates by trip were used, estimated number of ill persons ranged from 294 to 1056 (not including secondary cases, independent travelers, or business complex and bus company employees).

Environmental Investigation

There were 2 shallow wells at the busi-
Figure 1.—Cases of gastroenteritis in tour bus passengers, employees of tour bus company A, and employees of the implicated business complex, Yukon Territory and Alaska, June and July 1995. Ten persons (on bus trip 3) had onset on June 27 or 28; to place them in the Figure, 5 were assigned to June 26 and 27 and 5 were assigned to June 28 and 29. Eight employees of the implicated business had no specific recollection of when their illness began during the period; none was placed in the Figure.

Table 3.—Number of Hours Between Meals and Mean Onset Time of Illness, by Itinerary, Alaska and Yukon Territory, June 1995

<table>
<thead>
<tr>
<th>Trip No.</th>
<th>Itinerary</th>
<th>No. of Cases</th>
<th>Mean Time of Illness Onset</th>
<th>Skagway</th>
<th>Whitehorse</th>
<th>Implicated Restaurant</th>
<th>Beaver Creek</th>
<th>Fairbanks</th>
<th>Glennallen</th>
<th>Valdez</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3,6</td>
<td>Skagway to Fairbanks</td>
<td>36</td>
<td>9 PM</td>
<td>(B)63, (L)58</td>
<td>(D)51, (B)40</td>
<td>(L)33</td>
<td>(D)27, (B)16</td>
<td>(L)7</td>
<td>(D)2</td>
<td>...</td>
</tr>
<tr>
<td>2,4</td>
<td>Skagway to Valdez</td>
<td>15</td>
<td>12 AM</td>
<td>(B)66, (L)61</td>
<td>(D)54, (B)43</td>
<td>(L)36</td>
<td>(D)30, (B)19</td>
<td>...</td>
<td>...</td>
<td>(D)5</td>
</tr>
<tr>
<td>5</td>
<td>Fairbanks to Skagway</td>
<td>19</td>
<td>10 PM</td>
<td>(D)3, (L)10</td>
<td>(B)17, (D)28</td>
<td>(L)34</td>
<td>(B)41, (D)52</td>
<td>(L)59</td>
<td>(B)64</td>
<td>...</td>
</tr>
</tbody>
</table>

*(B), (L), or (D) prior to the number of hours between a meal and illness onset refer to breakfast, lunch, and dinner, respectively.
† Trip numbers correspond to Table 1.
‡ Mean onset time was rounded to the nearest hour and the date of travel was ignored. For details of how means were determined, see text.

Table 4.—Item-Specific Attack Rates for Gastrointestinal Illness in 153 Tour Bus Passengers, Yukon Territory, June and July 1995

<table>
<thead>
<tr>
<th>Item</th>
<th>Ate Did Not Eat</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>III Not III</td>
<td>III Not III</td>
</tr>
<tr>
<td>Any sandwich</td>
<td>34 (55)</td>
<td>17 (47)</td>
</tr>
<tr>
<td>Any soup</td>
<td>15 (33)</td>
<td>36 (69)</td>
</tr>
<tr>
<td>Any stew</td>
<td>15 (24)</td>
<td>35 (79)</td>
</tr>
<tr>
<td>Chill</td>
<td>12 (20)</td>
<td>39 (82)</td>
</tr>
<tr>
<td>Any dessert</td>
<td>15 (43)</td>
<td>36 (59)</td>
</tr>
<tr>
<td>Water</td>
<td>39 (40)</td>
<td>11 (60)</td>
</tr>
</tbody>
</table>

*Detailed food histories were obtained for passengers on bus trip numbers 4-6, 9, and 10 (see Table 1). OR indicates odds ratio; and CI, confidence interval.

Laboratory Investigation

Stool cultures for bacterial pathogens were negative for the initial 9 specimens; electron microscopy revealed SRSVs or small round viruses in 8 of the subse-

complicated but before the outbreak vehicle was determined, the restaurant voluntarily closed. Because a health inspection on July 1 found no major deficiencies, the restaurant was allowed to re-open. On July 12, the restaurant closed, pending sanitary improvements. Water samples collected on July 19 from the restaurant and other sites served by well 1 showed evidence of bacterial contamination (total and fecal coliform counts ranged from 10-50 colonies per 100 mL and 2-18 colonies per 100 mL, respectively). A sample from well 2 was negative for bacterial contamination. An engineering survey determined that well 1 was probably fed by lake water that passed through the septic pit before reaching the well (N. A. Jacobsen, P Eng, written communication, May 30, 1997). Fluorescein was visually and chemically detected in well 1 about 24 hours after being flushed into the septic pit.
quently 17 specimens. The 8 were from 7 passengers (3 from before the initial restaurant closure and 4 from after reopening; 6 were primary cases, 1 was a secondary case). Reverse transcription polymerase chain reaction and Southern hybridization analyses detected a SRSV strain belonging to genotype 2/P2B in 4 of 6 specimens from ill passengers eating at the restaurant before initial closure and 7 of 8 specimens from after its reopening. Nucleotide sequences of amplified products of 1 specimen from before closure and 1 after reopening were identical. A stool specimen collected from a restaurant employee was positive for genotype 2/P2B SRSV. Nucleotide sequence of the RT-PCR product from this specimen was identical to the 2 sequences obtained from ill passengers, demonstrating that virus found in the restaurant employee matched that of the ill passengers.

Two water samples from well 1 and 1 from well 2 were examined. No amplified products associated with SRSV were detectable by RT-PCR from the first or second concentrates. However, nested PCRs of well 1 first and second concentrates generated 87 base pair-amplified products. Sequenced products were identical to the corresponding portion of SRSV genome amplified from stool specimens from the 2 tourists and 1 employee (Figure 2). Except for trace amounts of primer dimer, no amplified products were generated from first or second concentrates of the well 2 sample or controls.

**COMMENT**

This investigation confirmed that a contaminated well supplying a restaurant caused an outbreak of SRSV gastrointestinal illness. Prior to recognition of the outbreak among bus passengers, there was illness in persons at the business complex. Four ill persons lived in a building connected to a septic pit contaminating the well and became ill on June 13 and 14. The outbreak in passengers began on June 20, if not earlier, consistent with the appearance of contamination of the well. Severity of illness among the tourists affected by the outbreak was reflected in need for hospitalization of a minimum of 6 persons.

The investigation was complicated by daily movement of travelers, making it impractical to conduct more than a single, relatively brief interview. However, occurrence of illness on different itineraries allowed us to identify the outbreak source without initial knowledge of etiology or vehicle. Since affected persons had eaten together at multiple establishments during 3 days prior to illness onset, to find the probable source, we compiled meal times for each itinerary, calculated number of hours between each meal and the mean time of illness onset by itinerary, and examined time intervals to see if they "crossed." To our knowledge, this method of "epidemiologic triangulation" has not been described.

After identifying the likely source, passengers were asked more detailed questions about foods and beverages consumed at the restaurant. This line of inquiry indicated that water was the likely vehicle. Investigation at the restaurant found illness among employees and identified the contaminated well. New illness among bus company employees stopped when the company ceased patronizing the restaurant. New illness in restaurant employees was not identified after June 29, possibly due to decreased exposure (less business volume) or immunity. We do not have data on their water consumption. Newly developed techniques confirmed the presence of SRSVs in well water. We believe this is the first investigation to successfully use such methodology. Risk of laboratory cross-contamination was reduced by examining stool and water at different laboratories and, at the laboratory where both were tested, by not sending amplification products generated from stool until water testing was completed. Finally, the outbreak cause and vehicle were established by molecular analysis showing SRSV genetic material found in water from the restaurant matched that of virus found in stools of ill bus passengers and an ill restaurant employee.

This investigation underscores the importance of fundamental public health practices such as restaurant inspection and assurance of safe water supplies. The large number of persons in rapid international travel increases likelihood of acquiring infection in 1 country and becoming ill in another and demonstrates the need for good communication and close working relationships between national public health agencies.

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