A Large, Multiple-Restaurant Outbreak of Infection with *Shigella flexneri* Serotype 2a Traced to Tomatoes


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**Background.** Foodborne outbreaks of *Shigella* infection are uncommon and tomatoes are an unusual vehicle. We describe a large, multiple-restaurant outbreak of *Shigella flexneri* serotype 2a infection that was associated with tomatoes.

**Methods.** We conducted nationwide surveillance and a case-control study, collected fecal specimens for culture, and measured the survival of the outbreak strain of *S. flexneri* in tomatoes.

**Results.** We interviewed 306 of 886 ill restaurant patrons and 167 control subjects. Matched univariate analysis showed that several food items were associated with illness, but only tomatoes remained significant in multivariate models. Illness peaked at each restaurant within 24 h after the arrival of hand-sorted bruised and overripe tomatoes from a new distributor; all patient isolates that were tested were indistinguishable by PFGE. Sliced tomatoes from the distributor were inoculated with the outbreak strain, and viable *S. flexneri* were recovered for 72 h.

**Conclusion.** To prevent such outbreaks, persons with shigellosis should be excluded from handling food at all points along the distribution chain.

Unlike *Salmonella*, *Shigella* species are infrequently recognized as a cause of foodborne disease outbreaks; only 172 (1.3%) of 13,173 foodborne disease outbreaks reported to the Centers for Disease Control and Prevention (CDC) from 1973 to 1997 were caused by *Shigella* species [1–3]. *Shigella flexneri* commonly causes diarrhea in the developing world but causes it less commonly in the United States, where *Shigella sonnei* predominates. From 1989 to 2002, *S. flexneri* accounted for 18.4% of *Shigella* isolates submitted to CDC [4]. From 1973 to 1999, only 49 *S. flexneri*-associated outbreaks of foodborne disease were reported (CDC, unpublished data).

On 31 May 2001, the Nassau County Department of Health (NCDH) in New York state notified the CDC of a large outbreak of gastrointestinal illness involving 5 local restaurants (hereafter referred to as restaurants A–E) under the same ownership. They reported culture-confirmed *S. flexneri* infection in 4 persons, all of whom had eaten at restaurant A before onset of illness. Subsequently, a nurse reported diarrhea in 19 of 70 persons who ate a hospital lunch catered by restaurant A on 24 May. Reports of illness in persons who had eaten at 4 other local restaurants (restaurants B–E) followed. The NCDH inspected the restaurants, obtained samples of prepared food, and destroyed the remaining food on 30 May. Workers were asked about illness and were required to submit a stool sample for culture. *S. flexneri* was isolated from the feces of 2 ill and 14 asymptomatic employees, and they were excluded from work. On 2 June, a team from the CDC arrived to evaluate the extent of the outbreak, identify the vehicle and source of contamination, and implement prevention and control measures.
SUBJECTS, MATERIALS, AND METHODS

Epidemiologic investigation. Information from restaurant patrons who reported illness to the NCDH was recorded, and active hospital and laboratory surveillance for suspected S. flexneri infection was established. All 50 state health departments and public health laboratories were alerted to identify additional outbreaks. Emergency department physicians, internists, and rheumatologists in Nassau County were asked to report cases of diarrhea-associated reactive arthritis syndrome.

To form hypotheses about likely food vehicles, we interviewed members of the hospital cohort who ate at restaurant A’s catered lunch on 24 May and others who ate lunch at the restaurant that day. We interviewed the restaurant owner to identify the ingredients of and the preparative procedures for uncooked menu items and to learn how all items, raw and cooked, were served.

We constructed restaurant-specific epidemic curves. To estimate restaurant-specific attack rates, we divided the estimated number of illnesses reported to the NCDH by the number of meals served at each restaurant during the outbreak.

In our case-control study, we preferentially selected individuals for telephone interview who had eaten early during the outbreak and whose meal party contained both ill and well members. To ensure accurate selection of control subjects matched by date of meal and restaurant, we excluded case subjects who had eaten >1 meal at any of the restaurants in the 4 days before illness, those who could not remember the entree they ate, and those with onset of diarrhea >4 days after their meal. We defined a confirmed case of diarrhea as a subject who reported having ≥3 loose stools in 24 h within 4 days after a meal eaten at an implicated restaurant between 22–28 May and from whom S. flexneri was isolated. A probable case was defined as a subject with similar symptoms from whom S. flexneri was not isolated. We used case interviewees to identify controls, who were meal companions who did not develop diarrhea. We defined a secondary case as a contact of a case who developed diarrhea and either did not eat at restaurants, who were meal companions who did not develop

Laboratory investigation. We reviewed laboratory reports of all S. flexneri infections that were submitted to the NCDH. The food samples that were collected were sent to the New York State Department of Health for culture. We cultured feces samples from restaurant employees and assessed the susceptibility of S. flexneri isolates according to NCCLS methods. Isolates recovered from samples from the catered lunch attendees and a random sample of isolates recovered from restaurant patrons were compared by PFGE at the New York State Department of Health according to PulseNet protocols. Restricted bacterial DNA was separated by electrophoresis on a CHEF Mapper system. The restriction endonucleases used were XbaI and AvrII. The PFGE banding patterns were interpreted by standard criteria. The New York State Department of Health confirmed the serogroup and serotype of the outbreak strain by slide agglutination with specific antisera. To assess the survival of S. flexneri serotype 2a in tomatoes, the outbreak strain was inoculated (6 x 10^4 colony forming units per gram) in quadruplicate into 25-g portions of sliced, damaged tomatoes inside tissue homogenizer bags and incubated for 72 h at room temperature. Uninoculated tomatoes served as negative controls. At 0, 24, 48, and 72 h, samples were homogenized for 2 min, and serially diluted aliquots were plated onto Endo and Hektoen enteric agars for incubation overnight at 37°C. S. flexneri 2a was identified by standard procedures.

RESULTS

Epidemiologic investigation. More than 1500 persons called the NCDH during the outbreak to report 886 illnesses. The NCDH received reports of 117 fecal cultures that grew S. flexneri; 116 were associated with the outbreak. No other outbreaks of S. flexneri infection were reported. No cases of arthropathy following Shigella infection were reported.

Hypothesis-generating interview responses suggested Greek salad and yogurt sauce were possible food vehicles. Illnesses were associated with meals consumed from 22–28 May 2001 variables. Variables significant in the final reduced model were fitted in multiple multivariable models to estimate risk factors specific for day (matching by restaurant), for restaurant (matching by day), and for day and restaurant. To find the most robust and parsimonious model, we stratified both by frequency matching (cases to controls within a meal party and by day and restaurant) and by individual matching (randomly selected case to control). We used Levin’s population-attributable risk to calculate the proportion of illness explained by the implicated vehicle.
Outbreak of Shigella flexneri 2a Infection

The attack rate varied by restaurant and by day. The 2 busiest restaurants, restaurants A and B, had served 244 (80%) of the interviewees and had the highest attack rates. The overall estimated attack rate for the 5 restaurants was 8%; however, 64% of patrons who ate at restaurant A on 23 May and 47% who ate at restaurant B on 24 May reported illness (figure 1); rates on other days ranged from 0%–20%.

We interviewed 306 (35%) of 886 ill patrons, including 50% who reported illness during the first 4 days of the outbreak, and 167 healthy meal companions. We identified 29 secondary cases. One hundred six cases (35%) and 67 controls (40%) were men. The median ages of the cases (45 years; interquartile range, 33–57 years) and controls (47 years; interquartile range, 26–59 years) were similar. Most of those interviewed (422 [89%]) ate an implicated restaurant meal between 22 and 25 May; 300 (63%) ate dinner.

The median duration of diarrhea was 5 days (range, 1–20 days); 111 (36%) reported bloody diarrhea. Other symptoms reported included stomach cramps (275 persons [90%]), headache (177 persons [58%]), fever (165 persons [54%]), myalgia (162 persons [53%]), and vomiting (57 persons [19%]). One hundred twenty persons (39%) reported missing work for a median of 2.5 days. Two hundred four persons (67%) saw a health care provider. One hundred eighty-one persons (59%) took an antibiotic, and 123 (40%) took an antimotility agent, including 55 (50%) who reported bloody diarrhea. Twenty-two persons (7%) were hospitalized (median duration, 3 days). No one died.

Of the ill patrons interviewed, 153 (50%) reported submitting a stool culture. Forty-nine cultures were positive for S. flexneri 2a, including 21 cultures of samples from persons who reported having taken an antibiotic (23%), compared with 28 cultures of samples from those who did not (43%). Persons with culture-confirmed cases were more likely than those with probable cases to report having diarrhea longer (median 7 days vs. 5 days), missing more work (median 5 days vs. 2 days), taking an antibiotic (96% vs. 52%), seeking medical care (88% vs. 63%), being hospitalized (16% vs. 9%), and, if hospitalized, having a longer hospital stay (4 days vs. 2 days). They also more frequently reported taking an antimotility agent for bloody diarrhea (68% vs. 51%), although overall use of antimotility agents was similar (52% vs. 60% of subjects).

Univariate analysis of exposures reported by 306 ill and 167 healthy persons for 132 food items at restaurants A–E (matched by day and restaurant) found several exposures to be significantly associated with illness (P < .05). Although exposures to tomatoes and to yogurt sauce were significant in one multivariable model, only exposure to tomatoes remained significant in all reduced models (table 1). The ingestion of tomatoes contaminated with S. flexneri accounted for more illness during the first 3 days of the outbreak (59% of cases on 22 May, 52% on 23 May, and 49% on 24 May) than did ingestion of yogurt sauce (23% of cases on 22 May, 26% on 23 May, and 39% on 24 May).

Only 2 raw food items, salad base and yogurt sauce containing hand-peeled cucumbers, were prepared in restaurant A and distributed to restaurants B–E. At each restaurant, toma-
Table 1. Comparison of reported illness due to *Shigella flexneri* serotype 2a according to food exposure and matched by day and restaurant, Nassau County, New York, May 2001.

<table>
<thead>
<tr>
<th>Food items consumed by subjects at restaurants A–E</th>
<th>No. of subjects who became ill/no. exposed (%)</th>
<th>Matched OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salad base</td>
<td>274/283 (97)</td>
<td>2.07 (0.76–5.66)</td>
</tr>
<tr>
<td>Cucumbers</td>
<td>99/260 (38)</td>
<td>1.78 (1.13–2.81)</td>
</tr>
<tr>
<td>Kebab platter</td>
<td>105/276 (38)</td>
<td>0.69 (0.46–1.04)</td>
</tr>
<tr>
<td>Pita sandwich</td>
<td>65/272 (24)</td>
<td>1.61 (0.94–2.78)</td>
</tr>
<tr>
<td>Feta</td>
<td>116/293 (40)</td>
<td>1.44 (0.95–2.20)</td>
</tr>
<tr>
<td>Grilled onions</td>
<td>31/285 (11)</td>
<td>1.76 (0.85–3.65)</td>
</tr>
<tr>
<td>Green pepper</td>
<td>73/227 (32)</td>
<td>1.47 (0.90–2.41)</td>
</tr>
<tr>
<td>Mushroom</td>
<td>51/272 (19)</td>
<td>1.78 (0.99, 3.22)</td>
</tr>
<tr>
<td>Olives</td>
<td>100/273 (37)</td>
<td>1.47 (0.94–2.29)</td>
</tr>
<tr>
<td>Parsley</td>
<td>76/228 (33)</td>
<td>3.06 (1.70–5.50)</td>
</tr>
<tr>
<td>Pita bread</td>
<td>249/296 (84)</td>
<td>1.84 (1.13–3.00)</td>
</tr>
<tr>
<td>Raw red onions</td>
<td>32/288 (11)</td>
<td>3.18 (1.27–7.95)</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>201/267 (75)</td>
<td>3.17 (2.05–4.90)</td>
</tr>
<tr>
<td>Red onion sauce</td>
<td>81/289 (28)</td>
<td>1.63 (0.99–2.67)</td>
</tr>
<tr>
<td>Yogurt</td>
<td>167/293 (57)</td>
<td>2.38 (1.58–3.59)</td>
</tr>
</tbody>
</table>

* a Reduced model OR, 2.76; 95% CI, 1.74–4.37; P < .0001.

b Reduced model OR, 2.16; 95% CI, 1.36–3.43; P = .001.

Toes, cucumbers, and additional items for salad were chopped on the same board and then kept in a salad bar that was refrigerated from the bottom, but located next to a hot grill. Sliced tomatoes were kept in the bin closest to the grill.

During the week before the outbreak, all 5 restaurants received tomatoes from a new distributor (distributor X), which was a family-run business limited to tomatoes. The distributor bought them in Queens from a wholesale marketer who had received tomatoes from Florida during May 2001. Employees for distributor X hand-sorted purchased tomatoes according to size and color, packaged them in new boxes, and resold them locally. Days before the outbreak, distributor X sold overripe and bruised tomatoes at a discount to the 5 restaurants involved in the outbreak. On 22 May, thirty-five 20-pound boxes of these "special grade" tomatoes were delivered to restaurant A in the outbreak. On 22 May, thirty-five 20-pound boxes of these "special grade" tomatoes were delivered to restaurant A.

Employees at restaurant A, recent immigrants from the Caribbean, were not available for interviewing. Interviews with restaurant employees revealed that no one was ill before or at the start of the outbreak. All had eaten many items served at the restaurant, but only 1 worker, a salad server, reported illness (onset occurred on 25 May). Ready-to-eat foods were handled without gloves at the restaurants, and handwashing stations had neither soap nor towels. No employee worked at more than 1 restaurant.

**Laboratory investigation.** The NCDH received reports of 118 isolates of *S. flexneri* recovered from 886 patrons and 16 isolates recovered from 150 restaurant employees. All isolates were susceptible to trimethoprim-sulfamethoxazole but resistant to ampicillin, according to NCCLS testing criteria [7]. No food samples yielded *Shigella* isolates; however, the implicated tomatoes were not available for culture. The New York State Department of Health confirmed that the outbreak strain was *S. flexneri* serotype 2a. All 23 isolates from restaurant patrons and workers that underwent subtyping by PFGE using XbaI and AvrII restriction endonucleases were indistinguishable (figure 2). Seven *S. flexneri* isolates from New York recovered in early 2001 had 7 distinct PFGE patterns; none resembled the outbreak strain. The only reported match outside New York was an isolate from North Carolina that was submitted to PulseNet in April 2001; it was indistinguishable from the first PFGE digest pattern but different from the second. Lastly, viable *S. flexneri* 2a, with counts of colony-forming units similar to or greater than the original inoculum, were recovered from damaged tomatoes from distributor X at each time point assessed during the 72 h of incubation. *S. flexneri* 2a was never recovered from uninoculated tomatoes.

**DISCUSSION**

This is the largest documented foodborne outbreak of *S. flexneri* infection, and the first epidemiologically linked to tomatoes. We believe that tomatoes, contaminated most likely at a terminal distribution site, started the outbreak that involved 5 restaurants under a single owner. Consumption of tomatoes was the only exposure that remained significant in multiple multivariable models. Within 24 h after the arrival of hand-sorted, bruised "special grade" tomatoes from a new distributor, the rate of illness peaked at each restaurant. No other establishment received "special grade" tomatoes, and heightened surveillance did not detect the outbreak strain of *S. flexneri* 2a elsewhere, locally or nationally. These findings suggest that tomatoes contaminated at a final distribution site, rather than at a more central site of production, processing, or distribution, caused the outbreak.

Fresh tomatoes would seem an unlikely suspect, because before 2000 published reports included only 3 foodborne out-
breaks of diarrhea linked to tomatoes (1 caused by Salmonella enterica serotype Baildon, 1 caused by S. enterica serotype Javiana, and 1 caused by S. enterica serotype Montevideo) [11–13]. However, a recent report of 3 more outbreaks suggests an emerging problem [14]. In the outbreak we describe, multiple mishaps likely contributed. First, tomatoes distributed to the affected restaurants were overripe and therefore less acidic than younger ones [15]. Second, the tomatoes were exposed to heat during storage in the bin next to the grill; expression of virulence genes in S. flexneri 2a is triggered at 37°C [16]. Third, the pathogen may have entered through breaks in the skin or the stem scar of the damaged tomatoes [17]. Fourth, because the unwashed tomatoes were eaten uncooked, even organisms on the surface would have been ingested. Fifth, the implicated tomatoes were hand-sorted by individuals whose health at the time was uncertain. Importantly, after S. Montevideo and S. Javiana were traced to a common tomato commercial warehouse in 1991 and 1993 [17], a hazard analysis control points program identified sorting of individual tomatoes in a packing line as 1 of 3 critical control points for contamination [18]. Finally, the compatibility of the food vehicle and the pathogen was confirmed experimentally; extended viability of the outbreak strain of S. flexneri 2a in inoculated sliced tomatoes purchased from distributor X was demonstrated. Thus, the most likely scenario is that the tomatoes were contaminated by the hands of an infected sorter at distributor X. Thereafter, the combination of bruised and broken tomatoes, unrefrigerated transport, and storage of cut fresh tomatoes next to the grill may have allowed the organism to multiply on and in the unwashed tomatoes.

As fresh tomatoes are a rare vehicle for bacterial pathogens, so, too, are Shigella species uncommon foodborne pathogens. Of foodborne outbreaks in the United States with an identified bacterial etiology, most are caused by zoonotic Salmonella serotypes [2, 3], which contaminate poultry and other foods of animal origin and may lead to produce-associated outbreaks through cross-contamination. Contamination of food with Shigella is rare in countries with adequate hand hygiene and waste disposal. When outbreaks of Shigellosis infection occur, they are usually caused by S. sonnei, which largely replaced S. flexneri during the sanitary revolution [19]. Whereas S. sonnei has caused several recognized produce-associated outbreaks of infection in the United States through contaminated lettuce, salad, shredded cabbage, melon, and parsley [20–24] and in Europe through contaminated lettuce and imported “baby” corn [25–27], S. flexneri has caused only 3 outbreaks; 1 was traced to green onions [28] and 2 were traced to lettuce (CDC, unpublished data).

This outbreak’s notable high attack rate and size are consistent with the pathogenicity of Shigella species [29]. Whereas
10^3 or 10^4 Salmonella organisms are usually necessary to cause widespread illness in those exposed, as few as 10 Shigella organisms may suffice [30]. Thus, many more illnesses would result from food minimally contaminated with Shigella than from food equally contaminated with Salmonella. The high attack rate contributed to the outbreak's recognition and burden, which included nearly 900 reported illnesses, > 200 healthcare visits, and 22 hospitalizations. The frequent use of antimotility agents by patients with bloody diarrhea underscores the need for education about the risk of using these medications by those with invasive gut pathogens. The absence of reports of arthropathy following Shigella infection, despite heightened surveillance, suggests that the incidence was likely not more than the 0.1% reported in other outbreaks of S. flexneri infection [31, 32].

Many foodborne outbreaks are not recognized, even when they are large, because contamination early in distribution leads to widespread illness with a low attack rate that is indistinguishable from the background rate of illness. Outbreaks identified and investigated often remain unsolved; during the period 1993–1997, 68% of all reported foodborne outbreaks remained unexplained [3]. This outbreak illustrates several features that make produce-related outbreaks particularly challenging. First, subjects do not remember exposure to many produce items, such as those used in a salad or as garnish. Second, cross-contamination in the kitchen may make identification of the original contaminated vehicle difficult. Third, even if a vehicle is identified, rapid turnover of produce and ever-shifting patterns of distribution impair the ability to trace back and culture samples of the contaminated food. The implicated item is rarely available for investigation [33, 34]. This suggests that the burden of produce-borne illness is far greater than is recognized.

During the past 30 years in the United States, both the consumption of fresh produce and the number of countries from which it is imported have increased markedly. Mirroring these trends, the proportion of recognized foodborne outbreaks attributable to fresh produce rose from 2% to 8% between 1973 and 1991, and the mean size of those outbreaks has doubled [1–3, 35]. The outbreak we describe dramatically illustrates what happens when breakdowns in sanitation occur during produce handling and food preparation. Although tomatoes contaminated during sorting likely started the outbreak, cross-contamination from the unwashed hands of food handlers likely perpetuated it. Handwashing must occur both at distribution sites and at sites of food preparation. Given the low infectious dose of Shigella species, exclusion of workers until stool culture results are negative may be required if strict handwashing compliance cannot be assured. Given the emphasis on healthier nutrition and increased global importation of produce, the range of vehicles is likely to expand, and, with it, the need for heightened precautions at all steps from farm to table.

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