Outbreak of Enterotoxigenic Escherichia coli Infection with an Unusually Long Duration of Illness

Jonathan S. Yoder,1,2 Shawn Cesario,3 Victor Plotkin,3 Xinfang Ma,4 Kate Kelly-Shannon,2 and Mark S. Dworkin2

1Public Health Prevention Service, Office of Workforce and Career Development, Centers for Disease Control and Prevention, Atlanta, Georgia; and 2Division of Infectious Diseases, Illinois Department of Public Health, Chicago, 3Lake County Health Department, Waukegan, and 4Division of Laboratories, Illinois Department of Public Health, Springfield, Illinois

(See the editorial commentary by Mintz on pages 1518–20)

Background. Enterotoxigenic Escherichia coli (ETEC) is an emerging cause of foodborne outbreaks of infection in the United States, yet its epidemiology is not completely understood.

Methods. In September 2004, we investigated an outbreak of infection due to ETEC at an Illinois corporation following a meal served to ~700 employees. Clinical samples were negative for enteric pathogens and were tested for ETEC using stool culture and polymerase chain reaction (PCR). An environmental investigation was conducted to determine whether food-service practices or conditions led to this outbreak. A case of illness caused by ETEC was defined as onset of diarrhea and ≥1 of the following symptoms during 23–30 September 2004: cramps, vomiting, nausea, headache, or weight loss.

Results. The 111 ill employees interviewed had only 1 meal in common. Cucumber salad and noodle salad from that meal were associated with illness; no food was available for testing. A PCR test for ETEC in stool was positive in samples from 6 of 11 patients; 3 ETEC serotypes were detected. The environmental investigation revealed no critical violations. The median duration of diarrhea (7 days) was longer than that observed for the majority of outbreaks of ETEC infection (4 days) and was associated with consumption of carbonated beverages (odds ratio, 4.5; 95% confidence interval, 2.0–10.3).

Conclusions. Emerging features of ETEC identified in this outbreak include the organism’s role in domestic outbreaks and its ability to cause prolonged diarrheal illness. Additionally, integrating the results of nonculture-based diagnostic techniques into foodborne outbreak surveillance presents challenges under the current guidelines of the Centers for Disease Control and Prevention.
heal-labile and heat-stable enterotoxin genes, the samples can be cultured for serotyping, or the PCR probes can be applied directly to the samples. This article describes a large foodborne outbreak of ETEC infection that had a median duration of symptoms longer than that observed in 24 of the 25 published foodborne outbreaks of ETEC infection in the United States during 1975–2003 (excluding waterborne and cruise ship outbreaks) [2, 3] and a longer duration of illness than that previously described for ETEC (typically <5 days) [1, 4, 5]. Analysis of the data derived from the outbreak investigation allows for an examination of possible exposures that might be associated with increased illness duration and consideration of the limitations of the CDC case definition for outbreaks of ETEC infection.

METHODS

Background. On 30 September 2004, the Lake County (Illinois) Health Department (LCHD) received a call from the operator of a local food-service company who said that ~100 persons who had been served a meal on 23 September were ill with diarrhea. The implicated meal occurred at a local corporation where ~700 employees had been served a free lunch. The buffet-style lunch had been prepared in the onsite cafeteria kitchen and served by the company’s cafeteria staff. An investigation was conducted by LCHD and Illinois Department of Public Health (IDPH) staff to assess the extent of the outbreak and to determine risk factors for illness.

Epidemiological investigation. A list of corporate employees reporting gastrointestinal symptoms was requested. Local hospitals and laboratories were contacted to alert the employees to the outbreak and to determine whether they had detected an increase in diarrheal illness, and the National Retail Data Monitor (NRDM) was queried for regional sales data regarding antidiarrheal medications.

The company surveyed their employees using a facility-wide e-mail to identify how many employees had reported illness after the shared meal. LCHD and IDPH staff administered a questionnaire by telephone to self-reported ill employees that inquired about all foods and beverages served at the 23 September meal, the time the employee ate, and factors that might be related to increased diarrhea duration (e.g., the use of antidiarrheal medications and preexisting conditions), as well as other potential illness risk factors (e.g., travel history and eating other meals from the company cafeteria). Factors associated with prolonged diarrhea duration were assessed by comparing exposures of case-patients who had diarrhea for <7 days with case-patients who had diarrhea for ≥7 days. To assess the effect of drinking carbonated beverages, we grouped the exposures as “any carbonated beverage,” and then for each carbonated beverage, we conducted a restricted analysis that excluded case-patients who had drunk the other carbonated beverages. A case of illness caused by ETEC was defined as onset of diarrhea (≥3 loose stools in a 24-h period) and ≥1 of the following symptoms: cramps, vomiting, nausea, headache, or weight loss during 23–30 September 2004.

The company also provided a list of employees who had attended the lunch but who had not become ill. LCHD and IDPH staff contacted a random sample of these employees at work by telephone to recruit them as control subjects. The same questionnaire was administered to them, except that the section on symptoms was excluded after their case status was determined. Analysis of risk factors for being a case-patient was performed after 0.25 control subjects per case-patient were identified. This ratio was achieved after performing a power calculation [6]. Traditionally, a ratio of control subjects to case-patients of 1:1 is recommended; however, when resources are limited, statistically significant results can be achieved with a lower ratio if the strength of the association between the exposure and case status is substantial.

Environmental investigation. A sanitarian from the LCHD inspected the corporate facility on 30 September and used the LCHD protocol [7] for investigating foodborne outbreaks. The investigation focused on the food ingredients, food-preparation practices, health of food-service employees, and physical space in which the food had been prepared.

Laboratory investigation. Stool samples from 12 ill employees were tested for enteric pathogens (norovirus, Salmonella species, E. coli O157:H7, Shigella and Campylobacter species, and parasites) at local hospital laboratories before the investigation. Stool samples were collected from 6 other symptomatic employees on 7 October and sent to the IDPH laboratory and the CDC for specific ETEC testing. The CDC performs tests for ETEC using both PCR and culture and determines serotypes among isolates; the IDPH laboratory performs tests using multiplex PCR techniques [8]. All specimens were tested at the IDPH for norovirus using PCR. Five stool samples from local hospital laboratories that had tested negative for enteric pathogens were submitted to the IDPH for PCR testing and were then forwarded to the CDC for confirmation.

Statistical analysis. Because all meal attendees could not be interviewed, the outbreak was analyzed as a case-control study rather than as a cohort study. An epidemic curve was constructed, and food-specific attack rates were calculated. Univariate analysis, in which ORs were calculated and hypotheses were tested using the χ² test, was performed. ORs with 95% CIs that excluded 1.0, and P values <.05 were considered to be statistically significant. A multivariate logistic regression model was constructed for menu items that were significantly associated with illness in the univariate analysis. This analysis methodology was also employed to examine factors related to prolonged duration (≥7 days) of illness.
RESULTS

Epidemiological data analysis. The corporation’s facility-wide e-mail survey identified 240 employees who had been ill with gastrointestinal symptoms. These employees were contacted at work; 111 of them were able to be interviewed and met the case definition (figure 1), and 29 healthy employees served as control subjects. Among case-patients (table 1), 111 (100%) had diarrhea, 107 (97.3%) of 110 had abdominal cramps, 67 (60.4%) of 111 had nausea, 63 (58.3%) of 108 had excess gas, and 23 (21.1%) of 109 had vomiting. Twenty-two (20.8%) of 106 case-patients sought treatment from their health care providers, 6 (6.7%) of 89 were examined at an emergency room, and 2 (2.3%) of 88 were hospitalized. No deaths occurred. The median incubation period was 28.5 h (range, 6–93 h). Median duration of diarrhea was 7 days (range, 1 to >15 days); 15 (13.5%) of the 111 case-patients were continuing to have diarrhea at the time of the interview. The median duration of diarrhea among patients with laboratory-confirmed cases was 12 days. The median age of case-patients was 25 years (range, 21–64 years). The NRDM query detected no community-wide increase in gastrointestinal illness.

Eight food items and 5 beverages were served at the lunch. Univariate analysis identified brownies, cucumber salad, Asian crispy noodle salad, peanut sauce, and salad greens as being associated with illness; none of the beverages were associated. Stratified analysis identified the cucumber salad (OR, 6.4; 95% CI, 2.6–16.0) and the Asian crispy noodle salad (OR, 4.5; 95% CI, 1.6–12.5) as being statistically significantly associated with infection; infection occurred in 110 (85%) of 130 persons who reported eating either of these 2 items, compared with 1 (10%) of 10 persons who did not (OR, 49.5; 95% CI, 5.9–412.5). The ingredients of the cucumber salad were vinegar, paprika, sugar, fresh cucumbers, carrots, and red onions. The Asian crispy noodle salad contained soy sauce, oil, vinegar, honey, sesame seeds, cooked linguini, fresh peppers, squash, zucchini, pea pods, mushrooms, red onions, cilantro, green onions, and bok choy. These 2 salads had 1 fresh food ingredient in common, red onions, that might have been the initial source of food contamination. Alternatively, cross-contamination might have occurred if food preparation for these 2 menu items occurred in the same space or in the same serving bowls or if utensils were intermixed. Multivariate analysis revealed that, after adjustment, eating the cucumber salad or the Asian crispy noodle salad remained significant predictors of illness.

The variables were further analyzed for their association with duration of diarrhea. Demographic characteristics, use of over-the-counter antacids or antidiarrheal medications, and number of servings of menu items were not associated with either increased or decreased duration of diarrhea. Each menu item was also analyzed for its association with duration. Case-patients who had an increased duration (≥7 days) of diarrhea were more likely to report drinking carbonated beverages (OR, 4.5; 95% CI, 2.0–10.3; P = .0003) at the implicated meal. Increased duration of diarrhea occurred in 49 (70%) of 70 case-patients who drank carbonated drinks, compared with 14 (34%) of 41 who did not. When the carbonated beverages were examined in a stratified analysis, brand A (OR, 16.3; 95% CI, 3.0–88.9; P = .0003) and brand B (OR, undefined; 95% CI, undefined; P = .005) were statistically significantly associated with increased duration of diarrhea, whereas brand C (OR, 2.7; 95% CI, 0.9–8.4; P = .08) only approached statistical significance. Potential confounding factors (e.g., persons who drank carbonated beverages might have eaten more of the implicated salads) could not account for this association.

Environmental investigation. No critical violations of food-handling or food-preparation practices were observed during the inspection of the food-service facility. The implicated meal had been prepared and served by cafeteria employees. The inspection of the food-service area revealed that kitchen ice machines and food preparation areas were provided with properly sized air gaps to prevent backing up of sewage into the units, and no improper temperature maintenance was identified. Food from the meal had been eaten or discarded, and no unprepared food had been retained in the kitchen.

The 6 food-service employees who prepared and served the implicated meal were interviewed and denied having been ill with gastrointestinal symptoms before, during, or after the event, and they reported that none of them had eaten food from the implicated meal. No additional illnesses were reported after the inspection of the facility or from employees who had not eaten the implicated meal. No information on the source (country of origin) of the menu items was available at the time of inspection. Because a single food item was not implicated as the outbreak source, a traceback investigation was not initiated.

Laboratory findings. ETEC was detected by PCR or culture of stool specimens from 6 of the 11 case-patients who submitted...
samples for testing and whose specimens were still available for testing. Four of the 6 stool samples collected by the LCHD on 7 October were found to be positive by PCR at the IDPH laboratory; 2 of these samples were found to be culture positive at the CDC. Two of the 5 specimens sent from local laboratories were found to be positive by PCR at the IDPH; 1 of these specimens was suitable for culture at the CDC, where it tested positive. Culture results from the CDC revealed 3 different serotypes: E. coli O27:H7 producing heat-stable toxin, E. coli O159:H4 producing heat-labile toxin, and E. coli O6:H16 producing both heat-labile and heat-stable toxins. No other enteric pathogens were identified in the stool specimens tested at local hospital laboratories or the IDPH laboratory.

**DISCUSSION**

This investigation illustrates multiple emerging features of outbreaks of ETEC infection, including their occurrence in economically developed countries, the organism’s ability to cause relatively prolonged diarrheal illness, and the challenges of integrating the results of nonculture-based diagnostic techniques into foodborne outbreak surveillance under the current CDC guidelines.

The discovery of multiple serotypes of ETEC, the statistical association between cucumber and noodle salads and illness, and the lack of illness among the food-service workers supports the hypothesis that the source of the outbreak was human sewage contamination of a fresh ingredient of these salads during growth, harvest, or processing rather than contamination by an ill food-service worker at the point of food preparation. The isolation of multiple ETEC serotypes during outbreak investigations is an infrequent finding in domestic outbreaks, having been reported in only 4 (16%) of 25 foodborne outbreaks in the United States during 1975–2003 [2, 3]. In those 4 multiple-serotype outbreaks, the implicated sources were parsley [9], carrots [10], scallops [3], and sewage backup [11]. However, such outbreaks might be more readily identified if a sufficient number of stool samples are collected and properly processed.

Multiple factors likely contributed to the inability of the laboratory investigation to confirm this as an outbreak of ETEC infection according to CDC guidance [12]. Public health officials were notified of the outbreak >10 days after the initial onset of symptoms, which led to collection of relatively few stool samples, because the majority of cases had resolved. Additionally, the majority of stool samples that were sent for testing were collected >14 days after onset of symptoms. Results from previous outbreak investigations indicate that ETEC organisms are rapidly cleared from the stool, reducing the rate of organism recovery in stools collected >7 days after the onset of symptoms [13, 14]. In addition, the CDC laboratory did not isolate ETEC from all of the specimens in which ETEC had been detected by PCR at the IDPH laboratory. Further consideration should be given to making the CDC case definition less specific, so that it can more-accurately count outbreaks of infection due to multiple serotypes of ETEC. Additionally, use of PCR techniques alone as a method of confirming outbreaks of ETEC infection should be considered [8, 15].

The reported median duration of diarrhea in this outbreak was longer than that described in the majority of domestic foodborne outbreaks of ETEC infection and exceeded the expected illness duration (usually <5 days) typically cited in public health reference tools [1, 4, 5]. This was unexpected because the median age (25 years), socioeconomic status (all patients were employed), and health status (none of the patients were immunocompromised) indicate that this population was relatively healthy. It is possible that the clinical pattern of outbreaks due to multiple serotypes of ETEC is different from that of single-serotype outbreaks. The duration of illness reported in multiple-serotype ETEC outbreaks in the United States is similar (median, 7.5 days) to the prolonged illness duration observed in this outbreak [2, 3]. A multiple-serotype outbreak caused by the same 3 serotypes [9] identified by this investigation was the only domestic foodborne outbreak with a longer median duration of illness than the one described here. Interpretation of published data on duration of illness from reports of domestic outbreaks should include whether those data were limited by collection, at the time of interview, of data regarding duration of illness, that did not include following up of patients who were still ill to determine their actual duration of illness.

Drinking carbonated beverages was associated with increased duration of diarrhea in this outbreak. This is an unexpected finding, because ETEC was demonstrated in an in vitro study [16] to have poorer survival in carbonated beverages than in

---

**Table 1. Occurrence of signs and symptoms associated with a foodborne outbreak of enterotoxigenic Escherichia coli infection.**

<table>
<thead>
<tr>
<th>Sign or symptom</th>
<th>No. of case-patients with sign or symptom/no., responding to survey questions (%) (n = 111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>111/111 (100)*</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>107/110 (97.3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>67/111 (60.4)</td>
</tr>
<tr>
<td>Excess gas</td>
<td>63/108 (58.3)</td>
</tr>
<tr>
<td>Headache</td>
<td>58/109 (53.2)</td>
</tr>
<tr>
<td>Fever</td>
<td>31/105 (29.5)</td>
</tr>
<tr>
<td>Dark urine</td>
<td>30/104 (28.8)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>23/109 (21.1)</td>
</tr>
<tr>
<td>Physician visit</td>
<td>22/106 (20.8)</td>
</tr>
<tr>
<td>Bloody stool</td>
<td>7/101 (6.9)</td>
</tr>
<tr>
<td>Emergency department visit</td>
<td>6/89 (6.7)</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>2/88 (2.3)</td>
</tr>
</tbody>
</table>

*Diarrhea was included in the case definition.*

---

1516 • CID 2006:42 (1 June) • Yoder et al.
water or milk. The authors of that study attributed their finding to the low pH of carbonated beverages. The paradoxical finding that drinkers of carbonated beverages had a significantly greater chance of increased duration of diarrhea requires additional study to determine whether it is a reproducible finding and to determine its biologic mechanism of action. It is possible that an ingredient of these carbonated beverages influenced the survival or pathogenicity of ETEC. We cannot rule out that this finding was caused by chance; however, the magnitude of the OR (4.5) and limited P value (.0003) indicate that these findings are real. If this association is valid and duration of diarrhea can be affected by choice of rehydration beverage, this finding has potential implications for the choice of beverage recommended for travelers to ETEC-endemic areas who might become ill with diarrhea and rehydrate themselves with available carbonated beverages.

The duration and severity of illness caused by ETEC infection has also been reported to be dependent on host factors. In a study comparing ETEC infections among Mexican adults and US travelers, the average duration of diarrhea among travelers was significantly longer (94 h among US adults vs. 49 h among Mexican adults; \( P = .0004 \)) [17]. The study authors attributed this finding to the lack of exposure to this pathogen among the US travelers in their water or food, and therefore, these travelers had no partial gut immunity. Hence, when reporting duration of illness for ETEC infection, specifying whether the travelers had no partial gut immunity. Hence, when reporting duration of illness for ETEC infection, specifying whether the data derive from populations where infection with the organism is endemic is important. Our findings concerning risk factors associated with longer diarrhea duration are limited by the lack of information regarding the dose of ETEC organism ingested and host factors, such as decreased gastric acidity or previous ETEC infection.

We conclude that this was a foodborne outbreak caused by multiple ETEC serotypes. The outbreak investigation demonstrates the utility of using the CDC criteria (e.g., a ratio of multiple ETEC serotypes. The outbreak investigation demonstrates the utility of using the CDC criteria (e.g., a ratio of ETEC serotypes: \( H11091 \) and \( H11092 \)) in outbreaks of diar- eahal illness when routine laboratory testing fails to detect an enteral pathogen [2, 3]. When ETEC is suspected, \( \geq 10 \) to 15 stool samples should be sent to a laboratory that has the ability to test for ETEC, and additional samples should be stored in case they are needed to establish that multiple persons are infected with the same serotype. Because adequate numbers of stool samples are not always available, we recommend that consideration be given to making the CDC case definition [12] for an outbreak of ETEC infection less specific, so that it can include outbreaks due to multiple serotypes of ETEC when ETEC is the only organism identified in the outbreak.

Acknowledgments

We thank Roger Coffman, Lawrence Mackey, Eric Nystuen, Pamela Abdul-Hakim, Pamela Smith, Melinda Walker, Heather Anderson, Mark Carlson, Leslie Price-Robison, Adam Huffman, Dorian Robinson, Karnail Mudahar, and Stephanie Borchardt for assistance in investigating this outbreak; Michael Lynch for technical assistance; and Cheryl Bopp for laboratory assistance. We also recognize Douglas Passaro of the University of Illinois at Chicago School of Public Health, who died during the writing of this manuscript. During his lifetime, he assisted with data analysis and management of the University of Illinois at Chicago School of Public Health student epi corps. He was an enthusiastic teacher, friend, and colleague.

Potential conflicts of interest. All authors: no conflicts.

References


