Epidemic of Gastrointestinal Tract Infection Including Hemorrhagic Colitis Attributable to Shiga Toxin 1-producing Escherichia coli O118:H2 at a Junior High School in Japan

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ABSTRACT. Background. An epidemic of gastrointestinal disturbances related to food ingestion occurred at a junior high school in Komatsu, Japan, and was caused by specifically Shiga toxin (Stx) 1-producing Escherichia coli O118:H2, which has not been reported previously in humans. No outbreak of E coli-producing Stx 1 alone had occurred.

Methods. A total of 526 students and 35 adult staff members who ate the same food at lunch in the school were investigated. Questionnaires about food consumption at lunch were given to all 561 subjects as well as to clinics and hospitals that had treated 79 patients. Stool specimens from 525 subjects, and food, water, and environmental specimens, including cooking utensils, were collected in an attempt to identify the pathogen.

Results. A total of 126 subjects (22.5%) developed a diarrheal illness. The pathogen was isolated from the stool in 131 subjects, 49 of which were asymptomatic, and from a dipper. Salads served over several days were identified as high-risk from food analysis. Gastrointestinal symptoms resembled those associated with previous infections of Stx-producing E coli, but were mild. No cases of the hemolytic–uremic syndrome developed. Headache was present in 87 patients. Three patients underwent surgery for acute appendicitis during this epidemic. Four of five carriers had received an antibiotic effective against the pathogen.

Conclusions. This outbreak of E coli O118:H2 demonstrated the clinical and epidemiologic features of infection by E coli that produces Stx 1 alone. Infections with such organisms are being recognized increasingly, and the pattern of disease observed may differ from the pattern observed with E coli O157:H7. Pediatrics 1999;103(1). URL: http://www.pediatrics.org/cgi/content/full/103/1/2; Escherichia coli O118:H2, Shiga-toxin 1, outbreak.

ABBREVIATIONS. HUS, hemorrhagic–uremic syndrome; Stx, Shiga toxin; RPLA, reversed passive latex agglutination.
mailed to the 19 clinics and hospitals that were consulted by patients. It included questions about the date of onset of symptoms; date of consultation; specific symptoms; physical findings; results of laboratory tests used to evaluate the blood, urine and stool; and treatment administered. Of 79 such medical questionnaires, 75 were available for analysis.

**Microbiologic Investigation**

Between July 15 and 23, 1996, stool specimens were collected and examined for *Salmonella*, *Vibrio*, and *Staphylococcus*, using standard procedures. Representative stool specimens from 20 patients with diarrheal illness, including 5 patients with bloody diarrhea, also were examined for *Shigella*, *Campylobacter*, and *Yersinia*, using standard procedures. The first five colonies of *E coli* selected from MacConkey agar were serotyped using *E coli O* and *H* antisera, and sorbitol–MacConkey agar also was used in the routine screening for *E coli O157:H7*. These colonies of *E coli* also were examined for Stx 1 and Stx 2, using a reversed passive latex agglutination (RPLA) kit (Denka Seiken Co, Ltd, Tokyo, Japan).

Isolates of *E coli* were inoculated onto 10 mL of brain–heart infusion broth (Denka Seiken Co, Ltd) containing 900 μg of lincomycin. After overnight incubation at 37°C, colonies were inoculated to 1 mL of saline containing 5000 U of polymyxin B and shaken at 37°C for 30 minutes. The culture was centrifuged for 30 minutes at 3000 rpm. The supernatant was tested using an RPLA kit. The minimum leukocyte count of each isolate could detect 2 ng/mL of purified Stx. Representative specimens were reexamined for Stx by the polymerase-chain reaction procedure described previously. 

Isolates were sent to the National Institute of Health (Tokyo, Japan) for serotyping. Asymptomatic subjects exhibited Stx-producing *E coli* in their stools were defined as healthy shedders. Immunoglobulin M antibody for O118 lipopolysaccharide in the serum of two patients with appendicitis was measured at the National Children’s Medical Research Center (Tokyo, Japan). 

**RESULTS**

The findings obtained from this outbreak are summarized in Table 1. Of 561 subjects who were at risk for the infection, 241 (43.0%) were defined as symptomatic and 126 (22.5%) developed a diarrheal illness. Of these patients, 9 were hospitalized with severe symptoms (6 patients with bloody diarrhea and 3 patients with acute appendicitis). The number of students with symptoms significantly exceeded that of the adult staff members (239/526 [45.4%] vs 2/35 [5.7%]; *P < .005*), and the number of students with diarrheal illness also significantly exceeded that of the adult staff members (125/526 [23.8%] vs 1/35 [2.9%]; *P < .01*). A characteristic of this outbreak was the surprisingly high number of patients who complained of headache, but there was no significant difference in the frequency of headache in the culture-positive versus culture-negative subjects (23/131 [17.6%] vs 59/394 [15.0%]). The majority of patients with abdominal discomfort complained of cramping in the periumbilical area. Only 5 (4.0%) of the 126 patients with diarrheal illness experienced vomiting. The average number of nonbloody diarrhea episodes per day was 3.3 ± 2.2 (ranging from 1 to 15 stools per day), and that of bloody diarrhea was 6.4 ± 2.6 (ranging from 3 to 10 stools per day). A watery diarrhea of 1.6 ± 0.9 days of duration (ranging from 0 to 3 days of duration) was antecedent to the onset of bloody diarrhea, and their peak body temperature was <38.0°C. No adult staff members developed bloody diarrhea.

Table 2 shows a comparison of laboratory findings during the acute phase in patients with bloody and nonbloody diarrhea. A mild but significant increase of the absolute neutrophil count and decrease of the platelet count were noted in patients with bloody diarrhea. Serum C-reactive protein value and leukocyte count were normal to slightly elevated in the majority of these patients. No fragmentation of erythrocytes was observed on the blood smears. Urinalysis revealed hematuria and/or proteinuria in 6 of the 29 patients tested. No cases of the HUS developed during the epidemic.

Two patients underwent surgery for acute appendicitis on July 16 and July 18, 1996. These patients exhibited previous watery diarrhea and a change from abdominal cramping to continuous pain in the right lower quadrant. Fever was absent in these patients. Their respective laboratory values were maximum leukocyte, 9.1 × 10^9/L and 10.0 × 10^9/L (9.1 × 10^9/L and 10.0 × 10^9/L); and maximum C-reactive protein, <0.24 and 0.47 mg/dL (<2400 and 4700 μg/L). Macroscopic examination confirmed a hyperemic and swollen appendix in both patients. The ileocecal region also was involved in 1 patient, whereas serious ascites was seen in the other. Micro-

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**TABLE 1. Summary of Findings: Epidemic of Gastrointestinal Illness at a Junior High School in Japan**

<table>
<thead>
<tr>
<th>Category</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>People at risk</td>
<td>561</td>
</tr>
<tr>
<td>Students (12½ to 15½ y)</td>
<td>526/561 93.8</td>
</tr>
<tr>
<td>Staff</td>
<td>35/561 6.2</td>
</tr>
<tr>
<td>Symptoms or signs</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>241/561 43.0</td>
</tr>
<tr>
<td>Symptomatic with nonbloody diarrhea†</td>
<td>117/561 20.9</td>
</tr>
<tr>
<td>Symptomatic with bloody diarrhea+</td>
<td>9/561 1.6</td>
</tr>
<tr>
<td>Symptomatic without diarrhea‡</td>
<td>115/561 20.5</td>
</tr>
<tr>
<td>Headache</td>
<td>87/241 36.1</td>
</tr>
<tr>
<td>With diarrhea</td>
<td>44/87 50.6</td>
</tr>
<tr>
<td>With abdominal pain only</td>
<td>34/87 39.1</td>
</tr>
<tr>
<td>Without other symptom</td>
<td>9/87 10.3</td>
</tr>
<tr>
<td>High temperature (&gt;38.0°C)</td>
<td>5/241 2.1</td>
</tr>
<tr>
<td>Confirmed pathogen by RPLA</td>
<td>131/525 25.0</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>49/303 16.2</td>
</tr>
<tr>
<td>Symptomatic with nonbloody diarrhea</td>
<td>53/106 50.0</td>
</tr>
<tr>
<td>Symptomatic with bloody diarrhea</td>
<td>4/5 80.0</td>
</tr>
<tr>
<td>Symptomatic without diarrhea‡</td>
<td>25/111 21.6</td>
</tr>
</tbody>
</table>

*Nonbloody diarrhea with vomiting in 4 patients, headache in 40, and high temperature in 5.*
† Bloody diarrhea with headache in 4 patients.
‡ Abdominal pain, vomiting, and/or headache without diarrhea.
¶ Stool culture was performed in 525 of 561 subjects at risk.

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**Statistical Analysis**

Data are reported as means ± 1 standard deviation unit. The student’s *t* test was used to compare laboratory values. The *χ*^2^ test was used to test for differences in frequency distribution and proportion. The Yates’ corrected *χ*^2^ test was applied when the expected value for a cell was <5. A level of *P < .05* was accepted as statistically significant.
The number of patients is noted in the parentheses. CRP indicates C-reactive protein; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase. For conversion to SI units: leukocyte count, 1/μL = 1 x 10^9/L; hemoglobin, 1 g/dL = 0.155 mmol/L; platelet count, 1/μL = 1 x 10^12/L; CRP, 1 mg/dL = 1 x 10^6 μg/L; blood urea nitrogen, 1 mg/dL = 0.375 mmol urea/L; creatinine, 1 mg/dL = 88.4 μmol/L; LDH, AST, and ALT, 1U/L = 1U/L.

Although Stx-producing E. coli was not isolated from the samples of food and water, it was isolated by RPLA from stool specimens of 131 (25%) of 525 subjects whose stools were examined by culture, and the incidence of pathogen isolated from subjects with diarrheal illness was significantly higher than that for asymptomatic and symptomatic subjects without diarrhea (Table 1). Of 131 subjects found to be positive for Stx-producing E. coli, 57 (43.5%) developed a diarrheal illness, 25 (19.1%) were symptomatic without diarrhea, and 49 (37.4%) were asymptomatic. There was no significant difference in the incidence of detection of this pathogen in the students versus the adult staff members (127/490 [25.9%] vs 4/35 [11.2%]). The isolate was identified as E. coli serotype O118:H2. This isolate fermented sorbitol. Additional characterization of the strain, such as the ability to adhere to epithelial cells or possession of the eae gene, was not performed in this study.

Although Stx-producing E. coli was not isolated from the samples of food and water, it was isolated from a dipper and identified as O118:H2. The analysis of the food eaten by the culture-positive versus culture-negative subjects revealed that high-risk food items served as lunch were coleslaw salad (July 5, 1996; P < .005), chicken and cucumber with cold mustard sauce (July 8, 1996; P < .05), sour sauce salad (July 9, 1996; P < .025), egg salad (July 10, 1996; P < .005), and corn salad (July 11, 1996; P < .01). Other food items served as lunch were rice; bread; soup; packed sterile milk; and thoroughly cooked meat, fish, eggs, and vegetables. No subjects developed the infection at the 11 other schools that had served the same vegetables and other foods and used the same menu. Five cooks developed no gastrointestinal symptoms, and Stx-producing E. coli was not isolated from their stools.

Of 75 symptomatic patients who consulted a hospital or clinic, 56 received antimicrobial agents. New quinolones were used in 33 patients (norfloxacin, 17; enoxacin, 5; lomefloxacin, 4; levofloxacin, 4; tosufloxin, 2; and ciprofloxacin, 1); fosfomycin in 27; macrolides in 3 (clarithromycin 2 and josamycin 1); cephems in 3 (cefalog, cefetam piroxil, and cefuroxime axetil); and tetracyclines in 1 (minocycline). Eleven patients received two antibiotics in combination.

Table 3 shows the relationship between the isolation of pathogen from the stool and the duration of antibiotic treatment. Although effective agents were used in treating this pathogen, 9 of 15 patients still exhibited it 1 day after the administration of antibiotics. In fact, the pathogen was detected even after the administration of antibiotics for 2 or 3 days. Table 4 shows the results of reexamination of stools from 470 students and 32 adult staffs for Stx-producing E. coli. In the 49 asymptomatic subjects identified as healthy shedders by the first stool culture, the pathogen disappeared from their stools without treatment on days 28.6 ± 5.1 after the first culture. However, reexamination of the stools of symptomatic subjects on days 26.0 ± 5.4 after the
coli O26:H11-producing only Stx 1 occurred in a small outbreak (the number of patients was 6) of E coli. Attention has focused on Stx-producing E coli isolated from the stools of 5 carriers after the readministration of antibiotics. Stx-producing E coli had received effective antibiotics, exhibited Stx-producing infection may be much lower than that of E coli-producing both Stx 1 and 2 or Stx 2 alone in infants or in the elderly. On the other hand, although the prevalence of diarrheal illness and other symptoms was significantly higher in the students than in the adult staff members, there was no significant difference in the incidence of isolation of E coli O118:H2 in those groups. This suggests that young people of junior high school age are more susceptible to Stx-producing E coli than are adults.

This outbreak was unusual in that many patients complained of headache. Because of Stx being referred to as a neurotoxin, headache is considered to be an effect of Stx. However there is a possibility that the headaches were not related to the infection of this organism, because there was no significant difference in the frequency of headache in the culture-positive versus culture-negative subjects. Another interesting feature of this outbreak was the finding of 2 patients with acute appendicitis. The diagnosis was verified surgically in both patients. However, additional discussion about the indication for operation is necessary, because the pathologic findings of the appendix in these patients resembled those seen with hemorrhagic colitis caused by infection of Stx-producing E coli. Suppurative appendicitis was absent. Swelling of the appendix caused by Stx led to the symptoms of appendicitis. Appendicitis has likely occurred in other outbreaks and sporadic infections caused by Stx-producing E coli.

Although the apparent source of the primary infection was not identified, salad was considered to be a high-risk food in the analysis of the food eaten by the subjects. However, no subjects developed the gastrointestinal symptoms at the 11 other schools that had served the same vegetables as salad. The pathogen was isolated from a dipper used in this school. It was suspected that the infection may have been transmitted by placing uncooked, uncontaminated food in contaminated utensils. Undercooked, contaminated food was excluded as a source. Although the subjects affected in this outbreak were...
not infants or the elderly, and *E. coli* O118:H2 produced Stx 1 alone, the prevalence of the infection was distinctly high. This pathogen might have been consumed repeatedly in contaminated salads over several days.

The use of antibiotics for treating Stx-producing *E. coli* infection is controversial. Carter and colleagues reported that antibiotic therapy was associated with an increased risk of secondary infection and a poor prognosis. Karch and researchers demonstrated that incubating *E. coli* O157:H7 with subinhibitory concentrations of trimethoprim-sulfamethoxazole resulted in a 4-fold increase in intracellular Stx and up to a 256-fold increase in extracellular Stx. In the present outbreak, there was no evidence that the clinical course was exacerbated by the administration of antibiotics. However, it is questionable whether the antibiotics could eradicate the pathogen. Although this strain was susceptible to the antibiotics used in many clinics and hospitals, the pathogen was isolated from the stool of many patients even after the initiation of antibiotic therapy. Stool cultures performed ~1 month after the onset of outbreak indicated that the incidence of pathogen isolated from symptomatic subjects treated with antibiotics exceeded significantly that of symptomatic subjects not receiving antibiotic therapy. We considered that the number of carriers may be increased by antibiotic administration. However, Karch et al reported that 13% of patients with *E. coli* O157 infection who received no antibiotic treatment became carriers. These questions need to be answered to establish the appropriate treatment for Stx-producing *E. coli* infections.

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