Multistate Outbreak of *Escherichia coli* O157:H7 Infections Associated with Consumption of Fresh Spinach: United States, 2006

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**ABSTRACT**

During September to October, 2006, state and local health departments and the Centers for Disease Control and Prevention investigated a large, multistate outbreak of *Escherichia coli* O157:H7 infections. Case patients were interviewed regarding specific foods consumed and other possible exposures. *E. coli* O157:H7 strains isolated from human and food specimens were subtyped using pulsed-field gel electrophoresis and multiple-locus variable-number tandem repeat analyses (MLVA). Two hundred twenty-five cases (191 confirmed and 34 probable) were identified in 27 states; 116 (56%) case patients were hospitalized, 39 (19%) developed hemolytic uremic syndrome, and 5 (2%) died. Among 176 case patients who provided spinach brand information, 106 (91%) consumed bagged brand A. *E. coli* O157:H7 strains were isolated from 13 bags of brand A spinach collected from patients’ homes; isolates from 12 bags had the same MLVA pattern. Comprehensive epidemiologic and laboratory investigations associated this large multistate outbreak of *E. coli* O157:H7 infections with consumption of fresh bagged spinach. MLVA, as a supplement to pulsed-field gel electrophoresis genotyping of case patient isolates, was important to discern outbreak-related cases. This outbreak resulted in enhanced federal and industry guidance to improve the safety of leafy green vegetables and launched an independent collaborative approach to produce safety research in 2007.

Key words: Epidemiology; *Escherichia coli* O157:H7; Multiple-locus variable-number tandem repeat analyses; Outbreak; Pulsed-field gel electrophoresis; Spinach

*Escherichia coli* O157:H7 causes diarrhea, often bloody, and may result in hemolytic uremic syndrome (HUS) and death. It is commonly transmitted in food, including undercooked ground beef, raw produce, and unpasteurized milk (20).

On 8 September 2006, Wisconsin State Laboratory of Hygiene staff uploaded a PulseNet WebBoard message reporting seven Wisconsin patient *E. coli* O157:H7 isolates with indistinguishable pulsed-field gel electrophoresis (PFGE) patterns, which initially defined the outbreak strain (*XbaI* PFGE pattern EXHX01.0124). These patterns were submitted electronically to the national PulseNet database at the Centers for Disease Control and Prevention (CDC). On the same day the Wisconsin state epidemiologist notified CDC about three noncontiguous clusters of *E. coli* O157:H7 infections and five patients with HUS in Wisconsin who underwent plasma exchanges (25). The seven isolates were not all linked to these cases. On 12 September, the New Mexico state public health laboratory (PHL) reported patient isolates with PFGE patterns indistinguishable from those in Wisconsin.

On 13 September, Oregon Public Health Division officials notified CDC of a cluster of six recent *E. coli* O157:H7 infections. Because of a computer security problem, these were not yet matched to any out-of-state isolates. On 13 September, investigators in Oregon and Wisconsin reported to CDC that they independently identified raw fresh spinach sold in bags as the likely vehicle for the illnesses in both states. Based on binomial calculations (15), the proportion of cases reporting fresh spinach consumption was significantly greater than the proportion of well survey participants in FoodNet sites...
analyses on clinical isolates of epidemiologists, and the PulseNet WebBoard. State PHLs performed PFGE that is the CDC outbreak listserve for state and local epidemiologists. Epidemic Information Exchange secure communications network to September 2006.

E. coli O157:H7 infection caused by the outbreak strain, by state, August to September 2006.

reporting fresh spinach consumption (2). The U.S. Food and Drug Administration (FDA) and California Food and Drug Branch were immediately notified.

On 14 September, CDC held a teleconference with multistate health departments and the FDA: 50 patients from eight states with illness onsets during August or September 2006 had E. coli O157:H7 isolates with indistinguishable PFGE patterns isolated from clinical specimens. That evening, the FDA advised consumers nationwide not to eat fresh bagged spinach (24). This report describes the multistate outbreak investigation.

MATERIALS AND METHODS

Case finding. CDC notified public health officials in all states about the outbreak by using the Health Alert Network (3), the Epidemic Information Exchange secure communications network that is the CDC outbreak listserve for state and local epidemiologists, and the PulseNet WebBoard. State PHLs performed PFGE analyses on clinical isolates of E. coli O157:H7 and submitted results to the national database (PulseNet) at CDC, and state investigators notified CDC of case reports. The CDC PulseNet team provided frequent reports of locations and counts of E. coli O157:H7 isolates with the outbreak PFGE pattern.

Case investigations. Cases were defined as confirmed, probable, or secondary among persons residing in the United States, with illness onset, or E. coli O157:H7 culture date (if onset date not known), from 1 August to 1 October 2006. A confirmed case was defined as (i) culture-confirmed E. coli O157:H7 infection in a person whose isolate exhibited the outbreak genotype (XbaI PFGE pattern EXHX01.0124 for screening, MLVA pattern A for final counts) or (ii) diarrheal illness in a person whose isolate exhibited the outbreak genotype (MLVA pattern A). A probable case was defined as diarrheal illness in a person with (i) culture-confirmed E. coli O157:H7 infection, but the isolate was discarded, or nonculture evidence suggesting E. coli O157:H7 infection (i.e., detection of Shiga toxin in stool, HUS, or serologic testing demonstrating antibody to O157 lipopolysaccharide); and (ii) consumption of raw spinach during the 10 days before illness onset or having an epidemiologic link to a confirmed or probable case. A patient with illness compatible with E. coli O157:H7 infection and illness onset ≥24 h after illness onset of a confirmed case in the same household was defined as a secondary case. HUS was determined based on physician diagnosis because laboratory data related to HUS diagnosis were not obtained from most cases.

A questionnaire was developed to collect information regarding illness severity and details of spinach purchase and consumption (10 days before illness onset) including brand, lot code, Universal Product Code (UPC), “best-by” dates, and spinach type (e.g., baby, organic).

Laboratory investigations. Most E. coli O157:H7 isolates were sent from clinical laboratories to local PHLs for serotype confirmation and PFGE analyses (25). Almost all (96%) isolates from case patients were sent to CDC for serotype confirmation, toxin typing, other virulence factor characterization, and multiple-locus variable-number tandem repeat analyses (MLVA) (11). The outbreak genotype was defined as XbaI PFGE restriction pattern EXHX01.0124 and subsequently as MLVA pattern A. MLVA pattern A genotype was defined as the predominant MLVA pattern; by definition, isolates with this pattern could have up to three repeat unit differences at one locus or one repeat unit difference at two loci.

Leftover spinach samples collected from case patient homes were tested in state PHL, state departments of agriculture, FDA (16), or CDC laboratories. Testing methods varied among laboratories. Shiga toxin–producing E. coli (STEC) was isolated and identified by broth culture and immunomagnetic separation; CDC and some state laboratories used PCR assays for Shiga toxin detection (6). STEC was further characterized by serotype, PFGE, and virulence factors. All isolates underwent confirmation and MLVA genotyping at CDC.

Statistical analyses. Statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc, Cary, NC). Exposure rates were compared between patients with different MLVA types by using matched odds ratios with exact 95% confidence intervals.

RESULTS

Demographic and clinical characteristics. Initially, 200 cases were identified based on PFGE genotyping of case isolates and 5 more cases were identified based on the combination of diarrheal illness, consumption of fresh spinach, and culture of spinach from their homes that yielded E. coli O157 with the outbreak PFGE genotype. Fourteen cases were excluded because they did not meet the case definition with respect to MLVA results. One confirmed case was excluded because the patient was not a U.S. resident.

The final total of 191 confirmed cases included 5 secondary cases. Confirmed cases occurred among residents of 24 states (Fig. 1). Wisconsin (n = 49), Ohio (n = 24), and Utah (n = 19) reported the most confirmed cases. Thirty-four additional probable cases were reported among residents from 18 states, including 3 states with no reported confirmed cases.

Among confirmed case patients, 72% were female, 55% were hospitalized, 17% developed HUS, and 2% died. The
proportion of case patients who developed HUS was greatest among children aged less than 5 years (35%) and aged 5 to 17 years (28%). There was no difference in proportion of HUS among females (17%) and males (18%) ($P = 0.9$).

Clinical and demographic features of probable cases were comparable to confirmed cases. Of three confirmed case patients who died, two cases had HUS. Of two probable case patients who died, one had HUS (Table 1).

Among confirmed primary cases, the earliest illness onset was 20 August (Fig. 2), and the latest was 16 September, 2 days after the FDA’s initial consumer advisory. The peak of the outbreak (32% of confirmed cases) occurred from 30 August to 1 September.

**Spinach exposure information.** Among 176 confirmed primary case patients with completed questionnaires and with known symptom onset dates, 161 (91%) reported eating spinach during the 10 days before illness onset. Twelve patients (7%) denied spinach consumption and 3 (2%) did not know. Among 161 patients reporting spinach consumption, 135 (83%) reported eating bagged fresh spinach, including the patient with illness onset on 20 August. Of the 135 patients, 127 (94%) reported eating raw spinach, 2 (2%) ate only cooked spinach, and 12 (9%) ate both cooked and raw spinach.

**Spinach product information.** Spinach product was processed and deposited into retail bags that were sealed without modified atmosphere. Among 45 opened bags of fresh spinach obtained from patient homes in 14 states, label information and UPC codes were available for 42 bags; 34 (81%) identified brand A bagged spinach processed and packed by firm X, and 2 bags had UPC codes consistent

### Table 1. Clinical and epidemiologic characteristics of patients with confirmed and probable cases, United States, August to September 2006

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Confirmed</th>
<th>Probable</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>191</td>
<td>34</td>
<td>225</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>138 (72)</td>
<td>24 (71)</td>
<td>162 (72)</td>
</tr>
<tr>
<td>Median age (yr) (range)</td>
<td>27 (1–84)</td>
<td>21 (1–94)</td>
<td>27 (1–94)</td>
</tr>
<tr>
<td>Hospitalized, no. (%)</td>
<td>97 (55)</td>
<td>19 (63)</td>
<td>116 (56)</td>
</tr>
</tbody>
</table>

Proportion of confirmed and probable case patients with HUS by sex and age group (years), no./total (%)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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<tbody>
<tr>
<td>&lt;5</td>
<td>10/36 (28)</td>
<td>6/17 (35)</td>
<td>16/53 (31)</td>
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<tr>
<td>5–17</td>
<td>7/21 (33)</td>
<td>6/17 (35)</td>
<td>13/38 (34)</td>
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<tr>
<td>≥60</td>
<td>5/29 (17)</td>
<td>1/5 (20)</td>
<td>6/34 (18)</td>
</tr>
<tr>
<td>Total</td>
<td>29/170 (17)</td>
<td>10/30 (33)</td>
<td>39/200 (20)</td>
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*Based on available information on hospitalization.*

*Based on 170 confirmed case patients with nonmissing data for HUS, age, and sex. HUS was determined based on physician diagnosis because laboratory data were not obtained from most cases.

**FIGURE 2.** Number of confirmed cases (outbreak MLVA type A) with known date of illness onset ($n = 180$) of E. coli O157:H7 infection and MLVA types B to Q (nonoutbreak strains with the outbreak strain PFGE pattern), by date of illness onset, and median time interval of days from production of implicated lot of spinach to consumption, and the median incubation interval in days.
with brand B, also produced by firm X. Lot codes printed on the bags indicated the specific production facility, packaging date, work shift, and production line, but not all lot code information was legible or intact on recovered packages. Among 41 bags with lot code information, 30 (71%) had a lot code with the leading letter “P” (indicating the processing facility) and 17 (41%) had a Julian date of 227, indicating production on 15 August 2006, the earliest production date identified among collected packages.

Lot codes also indicated the daily production shift (A or B) and production line in the facility (one of four). Among 17 bags with Julian date 227, all were produced during shift A. Among 15 bags with available line codes, 7 (47%), 4 (27%), and 4 (27%) were produced on lines 01, 02, and 03, respectively. For patients who reported eating spinach only once and on a specific date, the median interval from production of lot 227 to consumption was 12 days (range, 2 to 25) and the median incubation period was 4 days (range, 1 to 14). Adding these median intervals resulted in an estimated median interval of 16 days from date of production of implicated spinach to the peak of the outbreak (Fig. 2). No confirmed case patient had illness onset before 20 August, 5 days after production of lot 227, which may fit within the time frame of earliest availability of and exposure to this lot of spinach.

Laboratory investigations: human isolate testing. During September 2006, the outbreak XbaI PFGE pattern accounted for 30% of all E. coli O157 patterns submitted to PulseNet, 10 times greater than during the same period in 2005. Using PCR testing, all isolates that matched the XbaI PFGE pattern EXHX01.0124, 197 (96%) were analyzed using MLVA. Of these 197 isolates, 183 (93%) isolates exhibited indistinguishable or highly related MLVA patterns designated as the pattern A series and 14 (7%) isolates had different MLVA patterns designated as patterns B through Q (Fig. 2).

Case patients with MLVA type A isolates were significantly more likely to have consumed spinach during the 10 days before illness onset than case patients with MLVA types B to Q isolates (156 [93%] of 168 versus 4 [36%] of 11 patients, odds ratio = 22.8, 95% confidence interval, 5 to 111).

Spinach product testing. Among 44 samples of bagged spinach from patients’ homes, 13 samples from 10 states yielded E. coli O157:H7 isolates with PFGE patterns indistinguishable from the outbreak pattern (Table 2). Eleven of these positive samples had lot code P227; the other 2 positive samples had no lot code information available. Case patients with bags of spinach that tested positive for O157:H7 had illness onset from 25 August to 7 September 2006. Twelve E. coli O157:H7 isolates from spinach samples were analyzed using MLVA; all 12 isolates demonstrated the MLVA type A pattern.

Two other STEC were identified in bags of fresh spinach: Orough:NM isolates were isolated from two samples from two states and had PFGE patterns distinct from the O157:H7 outbreak strain, but indistinguishable from each other and from the Orough:NM isolate from one confirmed case patient in Wisconsin. Neither of the E. coli Orough:NM–positive spinach samples were associated with the Wisconsin case patient. Two spinach samples from homes of patients with suspect cases (one in Maryland and one in Illinois) each yielded STEC O146:H21; the PFGE patterns of these isolates were indistinguishable. E. coli O157:H7 was not isolated from these spinach samples or from the probable case patients; PCR of the Orough:NM and O146:H21 isolates indicated the presence of stx2a and ehy, but not stx2b or eae. Stool samples from case patients who supplied these spinach samples did not yield O146:H21 or Orough:NM, although not all samples were tested to detect non–O157 STEC in general or O146:H21 or Orough:NM in particular.

DISCUSSION

During September and October 2006, public health officials investigated one of the largest outbreaks of E. coli O157:H7 infections in the United States, resulting from spinach consumption. Among 225 cases, 191 cases were confirmed and 34 cases were probable. Many illnesses were severe: 39 (19%) patients developed HUS and 5 patients died. The extent of this outbreak was likely greater: an estimated 26 cases occur for every E. coli O157:H7 reported case (21), suggesting that more than 5,000 infections were caused by one contaminated lot of spinach. Underrecognition of E. coli O157:H7 infections occurs because people may not seek medical attention; health care providers may not order stool testing; laboratories may not test for E. coli

<table>
<thead>
<tr>
<th>TABLE 2. Results of testing bags of fresh spinach obtained from case patient homes, United States, August to September 2006</th>
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<tbody>
<tr>
<td>States</td>
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</tr>
<tr>
<td>Arizona</td>
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<td>Colorado</td>
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<td>New Mexico</td>
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<td>Pennsylvania</td>
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<td>Wisconsin</td>
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<td>Nebraska</td>
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<tr>
<td>Utah</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Bags of spinach collected</td>
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<tr>
<td>Bags of spinach tested</td>
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<tr>
<td>E. coli O157 detected, no. of bags</td>
</tr>
<tr>
<td>Outbreak PFGE pattern detected, no. of bags</td>
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<tr>
<td>Outbreak MLVA pattern detected, no. of bags</td>
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</table>

a State (case patients): Arizona (1), Colorado (1), Illinois (1), New Mexico (1), Nevada (1), Ohio (1), Pennsylvania (2), Wisconsin (2), Nebraska (1), and Utah (2).

b One E. coli O157 strain isolated from spinach was not analyzed by MLVA.
O157:H7 (10); and when a diagnosis is made, the case may not be reported to public health officials or the isolate may not be forwarded for PFGE analysis in a PHL, participating in PulseNet. Additional serotypes of STEC detected among patients and spinach samples may have caused an unknown number of outbreak-related illnesses because these serotypes were often not identified in clinical microbiology laboratories (10). The role that STEC played in the infection and their potential sources are unclear.

Atypically high rates of HUS and mortality were detected during this outbreak. Generally, 6 to 8% of E. coli O157:H7 patients develop HUS, of which 5% die (7, 22). Observed illness severity was likely related to two factors. First, the outbreak strain produced only Shiga toxin 2. Such strains result in higher rates of HUS than strains that produce Shiga toxin 1 or both toxins (19). Greater virulence of the strain might be explained by the presence of two sets of stx2 genes, stx2A and a stx2C variant, that were found in outbreak isolates from California (clinical, spinach, and bovine) (12, 16, 26). Second, although the most-probable number method was not done, the high proportion of bagged spinach samples yielding the outbreak strain suggests high contamination levels of spinach. Third, spinach is a ready-to-eat food that does not have to be cooked before consumption, so the dose consumed might have been higher than in other vehicles that are usually at least partially cooked, such as hamburger.

Early outbreak recognition and investigation was facilitated by epidemiologists rapidly interviewing outbreak PFGE-matching patients by using wide-ranging, hypothesis-generating “shotgun” questionnaires. Investigators suspected fresh spinach as the likely source of infections within 1 week after outbreak detection based on a relatively small number of initial interviews.

Ultimately, epidemiologic and laboratory evidence presented in this report and from case control studies conducted in Wisconsin, Utah, and New Mexico (8, 25) confirmed that fresh spinach produced by one firm, at one facility, on 1 day, and distributed nationally was the vehicle of transmission. Data on the geographic distribution of residences of patients from which contaminated bags of spinach with the same lot code were obtained, and the epidemic curve of confirmed cases strongly suggests that this lot code was responsible for the majority of cases. Brand, lot code, and UPC code from bags of implicated spinach were instrumental in the traceback of the product to the firm X facility.

The median time from illness onset to PFGE pattern submission to PulseNet was 16 days, a time that was similar to the interval (15 days) noted among systematically selected cases of E. coli O157:H7 infections reported in six states during 2002 (9) and had not changed significantly in 2015 (CDC, personal communication). This timeline included time for ill persons to seek medical care; for health specialists to obtain a specimen; for clinical laboratories to culture, identify, and send the E. coli O157:H7 isolate to a PHL; for PHLs to conduct PFGE testing; and for electronic uploading of the PFGE pattern to PulseNet. This surveillance delay is inherent in the sequence of events required for E. coli O157:H7 isolation in clinical laboratories and PFGE analyses in public health laboratories.

To increase surveillance sensitivity, we initially defined cases based on PFGE results using the XbaI restriction enzyme only. Later on, as specificity became more important, application of MLVA distinguished 14 E. coli O157 PFGE-matched isolates as unrelated to the outbreak. Several of these excluded cases occurred in early August (Fig. 2). Excluding these cases established the outbreak onset in mid-August, corroborating exposure data identifying the implicated lot with a production date of 15 August and avoiding needless additional investigation into other lots or exposures responsible for PFGE-related cases in early August. Furthermore, patients with isolates demonstrating MLVA pattern A were significantly more likely to have reported spinach consumption during the 10 days before illness onset compared with patients with isolates demonstrating other MLVA patterns. MLVA offers additional advantage of high-throughput testing and easily portable digital pattern designations. With the recent advent of next-generation whole genome sequencing (WGS) technologies, researchers and public health and regulatory officials have begun using them for pathogen characterization with unprecedented genetic resolution. For example, the DNA sequence of the genome of the E. coli O157:H7 strain responsible for this outbreak has been analyzed to identify genes that may contribute to its virulence (17). The application of WGS to public health surveillance is especially promising. Two years of prospective surveillance for listeriosis by using WGS has demonstrated the ability to detect increased numbers of listeriosis clusters and to solve more outbreaks by using WGS compared with surveillance by using PFGE-based genotyping. WGS is being applied to other foodborne pathogens under public health surveillance, including STEC (13).

An extensive joint investigation was conducted by investigators from the California Department of Public Health, CDC, California Food and Drug Branch, and the FDA. They identified the source of the implicated lot as fields located on four ranches near the central California coast (5, 14). Although E. coli O157:H7 was isolated from environmental samples collected from all four locations, isolates matching the outbreak strain of E. coli O157:H7 were only isolated from environmental samples collected from one ranch. Spinach was grown on a leased portion of the ranch that was separated from cattle pastures by wire mesh fence (14). Samples from feral swine and cattle on this ranch yielded the MLVA outbreak strain; subtypes related by genotypes were isolated from surface water, sediment, and soil. There were multiple possible mechanisms of contamination of the spinach, including feral swine in and around the fields and the use of pelleted animal manure as fertilizer (14).

Other outbreaks of E. coli O157:H7 infections associated with consumption of leafy green produce have been traced to California fields. From 1995 to 2006, Cooley et al. (4) reported that nine outbreaks of E. coli O157:H7 infections were traced to, or near, the Salinas Valley region of California. These outbreaks have stimulated food safety agencies, industry, and academia to increase research and
prevention efforts (1, 18) to advance the knowledge of mechanisms of leafy greens contamination, the means by which E. coli O157:H7 adheres to or invades leafy greens (1), and methods to prevent or ameliorate contamination of leafy greens. In 2007, the California leafy greens industry was made responsible for self-regulation and enforcement of best production practices and use of an agreed upon traceback system (23).

This outbreak focused attention on the importance of strengthening enteric disease surveillance in all states, not only for the rapid investigation of outbreaks such as the outbreak described here, but also for the many smaller outbreaks that may go undetected or uninvestigated. This strengthened approach results in faster investigations by including routine submission and faster subtyping of isolates and more consistent submission of Shiga toxin–positive specimens by clinical laboratories to PHLs for primary isolation of STEC, including non–O157 serotypes; faster initiation of interviewing ill persons; and rapidly adaptable electronic data gathering and transmission platforms. The CDC, FDA, other federal and state agencies have been improving the efficiency of epidemiologic investigations through OutbreakNet, the national network of public health officials that investigates outbreaks of enteric illnesses, through the network of sites participating in the Foodborne Diseases Centers for Outbreak Response Enhancement (FoodCORE), and through the Council to Improve Foodborne Outbreak Response. FDA has created a multidisciplinary group (Coordinated Outbreak Response and Evaluation) to coordinate foodborne outbreaks investigations, and it has provided funding and technical assistance to 19 states and 32 state food laboratories to develop rapid response teams to conduct tracebacks and environmental investigations. The Food Safety Modernization Act provides the FDA with enhanced authority to implement prevention measures in the food production system. This outbreak investigation highlights the public health importance of integrating enteric pathogen surveillance with rapid outbreak response. Although it identified areas requiring further improvements, such as the time lag between specimen collection and PFGE results, it also demonstrated the value of using innovative laboratory tests in outbreak investigations.

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clades 6 and 8 of enterohemorrhagic Escherichia coli O157:H7 with particular stx subtypes are more frequently found in isolates from hemolytic uremic syndrome patients than from asymptomatic carriers. Open Forum Infect. Dis. 1:ofu061.


