A Multistate Outbreak of *Escherichia coli* O157:H7 Infections Associated With Consumption of Mesclun Lettuce

Elizabeth D. Hilborn, DVM, MPH; Jonathan H. Mermin, MD, MPH; Patricia A. Mshar, BS; James L. Hadler, MD, MPH; Andrew Voetsch, MPH; Christine Wojtkunski, MPH; Margaret Swartz, RN; Roger Mshar, MA; Mary-Anne Lambert-Fair, BS; Jeffrey A. Farrar, DVM, MPH; M. Kathleen Glynn, DVM, MPVM; Jonathan H. Mermin, MD, MPH

**Background:** An outbreak of *Escherichia coli* O157:H7 infections in Connecticut and Illinois during May 28 to June 27, 1996, was investigated to determine the source of infections.

**Methods:** Independent case-control studies were performed in both states. Pulsed-field gel electrophoresis (PFGE) was performed on *E coli* O157:H7 isolates. A case-patient was defined as a Connecticut or northern Illinois resident with diarrhea whose stool culture yielded *E coli* O157:H7 of the outbreak-associated PFGE subtype. Controls were town-, age-, and sex-matched to case-patients. We traced implicated lettuce to the farm level and performed environmental investigations to identify unsafe lettuce production practices.

**Results:** In Connecticut and Illinois, infection was associated with consumption of mesclun lettuce (Connecticut matched odds ratio [MOR], undefined; 95% confidence interval [CI], 3.4 to ∞; and Illinois MOR, undefined; 95% CI, 1.4 to ∞). We traced implicated lettuce to a single grower–processor. Cattle, a known *E coli* O157:H7 reservoir, were found near the lettuce fields. *Escherichia coli* (an indicator of fecal contamination) was cultured from wash water and finished lettuce. A traceback investigation identified 3 additional states that received implicated lettuce; *E coli* O157:H7 isolates from patients in 1 of these states matched the outbreak-associated PFGE subtype.

**Conclusions:** This multistate outbreak of *E coli* O157:H7 infections was associated with consumption of mesclun lettuce from a single producer. Molecular subtyping facilitated the epidemiological investigation. This investigation increased the knowledge about current production practices that may contribute to the contamination of lettuce by microbial pathogens. Lettuce production practices should be monitored for microbiological safety.

Arch Intern Med. 1999;159:1758-1764

*Sch. coli* O157:H7 is a leading cause of hemorrhagic colitis and childhood kidney failure. First recognized as a human pathogen in 1982,1 most reported foodborne outbreaks of *E coli* O157:H7 infection have been associated with foods of bovine origin, such as beef,2-4 dry-cured salami,5 and milk.6 Waterborne and person-to-person transmission also may occur.7,8 Recently, several outbreaks of *E coli* O157:H7 infections associated with consumption of raw fruit and vegetable products have been reported.10-16

In June 1996, state health department personnel in Connecticut and Illinois noted an increase in reported *E coli* O157:H7 infections; *E coli* O157:H7 infection is a reportable disease in both states. Molecular subtyping of patient isolates confirmed that the majority of reported infections in both states were caused by the same strain of *E coli* O157:H7. This article describes the investigation of a multistate outbreak of *E coli* O157:H7 infections associated with eating mesclun lettuce (a mixture of small red and green leaf lettuces) distributed by a single producer, stresses the need to develop production methods that minimize microbial contamination of lettuce, and emphasizes the value of molecular subtyping to link geographically separate outbreaks.

**LABORATORY ANALYSIS AND CASE FINDING**

Pulse-field gel electrophoresis subtyping was performed on 30 *E coli* O157:H7 isolates from Connecticut cases and 47 isolates from Illinois cases. Twenty-one patients from Connecticut and 28 patients from Illinois were infected with the outbreak-associated subtype. In addition, iso-
MATERIALS AND METHODS

CASE FINDING

On June 12, 1996, the Illinois state epidemiologist was notified of 7 laboratory-confirmed isolates of *E. coli* O157:H7 from persons living in the Chicago area. Only 2 cases had been reported during the same period in 1995. All local health departments and infection control nurses were notified of the increased number of cases and asked to report all cases to the Illinois Department of Public Health (IDPH).

On June 13, personnel from Foodborne Diseases Active Surveillance Network notified epidemiologists at the Connecticut Department of Public Health (CDPH) of 6 *E. coli* O157:H7 infections recently reported from laboratories in 2 counties under active surveillance. In contrast, no cases had been reported from these counties since January 1, 1996. Active case finding was subsequently expanded to include all Connecticut clinical laboratories. Laboratory personnel were asked to forward *E. coli* O157:H7 isolates to the CDPH. Public health officials in other states and the Centers for Disease Control and Prevention (CDC) were notified of the increase in reported cases and were requested to report cases of *E. coli* O157:H7 infection in patients who had recently visited Connecticut.

LABORATORY INVESTIGATION

*Escherichia coli* isolates were sent to the CDPH and IDPH for confirmation and determination of H7 antigen status. Confirmed isolates were forwarded to CDC for pulsed-field gel electrophoresis (PFGE) analysis. Pulsed-field gel electrophoresis was performed after digestion with *Xba*I (Boehringer Mannheim, Indianapolis, Ind), essentially as described previously.17 Selected strains from both states were also selected with *Spe*I to confirm the similarity of patterns with a different restriction enzyme.

CASE DEFINITION

A case was defined as diarrhea (≥3 loose stools in a 24-hour period) and a stool culture that yielded *E. coli* O157:H7 of the outbreak-associated PFGE subtype. Cases were restricted to Connecticut residents who had onset of illness during May 29 to June 27 and to residents of northern Illinois who had onset of illness during May 28 to June 21.

EPIDEMIOLOGICAL INVESTIGATION

Epidemiologic investigations were initiated and performed independently in Connecticut and Illinois. In Connecticut, all patients included in the case-control study were asked to identify 2 age- and sex-matched acquaintances who lived within the same town to act as controls. If a patient was unable to identify acquaintance controls, then the patient’s physician was asked to identify controls; alternatively, controls were selected by progressive, sequential-digit dialing within the patient’s town. During June 13 to July 5, patients and controls were interrogated by telephone with a standardized questionnaire. A potential control was excluded if ill with diarrhea during the 7 days before the matched case-patient’s onset of symptoms. Study participants were asked about recent illness and exposures during the 5 days before the onset of the patient’s illness. Data were collected about the study participant’s attendance at social events and restaurants, international travel, consumption of specific foods, and other exposures previously linked to *E. coli* O157:H7 infections.

In Illinois, open-ended hypothesis-generating interviews were conducted by telephone with 15 patients; exposures common to more than 50% of patients, or those exposures known to be risk factors for *E. coli* O157:H7 infection were included in a standardized questionnaire for the case-control study. Two age-, sex-, and telephone-exchange-matched controls were selected for each case-patient. Eligible controls had no history of diarrhea or abdominal cramping during the previous month. Telephone interviews were conducted from June 27 to July 2. Patients were asked about clinical symptoms, food and water consumption, and swimming during the 7 days before onset of illness; controls were questioned about the same exposures during the 7 days before the interview. A second questionnaire asking specific questions regarding lettuce consumption during the same periods as the first questionnaire was administered to patients and controls from July 29 to August 5.

LETTUCE TRACE-BACK INVESTIGATION

Separate trace-back investigations in Connecticut and Illinois focused on retail outlets (restaurants and grocery stores) that had sold mesclun lettuce to patients during May 20 to June 7. Retail invoices and bills of lading were requested. In each state, the lettuce was traced to the distributor level, where wholesale invoices were requested.

The producer of implicated lettuce was visited and investigated. Company personnel were interviewed regarding farming practices, processing methods, and transport.

LETTUCE TRACE-FORWARD INVESTIGATION

Customer information was obtained from the lettuce producer and used to identify states that received implicated mesclun lettuce. Health officials in those states were contacted and asked to perform PFGE analysis on isolates of *E. coli* O157:H7 collected during May to June to determine if they matched the outbreak-associated subtype.

ENVIRONMENTAL INVESTIGATION

Environmental samples were collected from lettuce-processing equipment, surfaces, and the surrounding environment using sterile swabs. Swabs were placed in Cary-Blair transport medium (Difco Laboratories, Detroit, Mich) and tested for *E. coli* O157:H7 at the California Department of Health Services Laboratory. Samples were analyzed by placing swabs in a selective enrichment broth, then seeded in an enzyme immunoassorbent assay (Assurance EIA- *E. coli* O157:H7; Bio Control Systems, Inc, Bothell, Wash). Water samples were taken from source well water, lettuce-processing water, and standing water in the local environment. The most probable number (MPN) of *E. coli* (not *E. coli* O157:H7) was assessed as an indicator of fecal contamination.18 Determination of *E. coli* MPN per 100-mL water sample was made using Colilert and Quanti-Tray (IDEXX Laboratories Inc, Westbrook, Me).

STATISTICAL ANALYSIS

Matched odds ratios (MORs) were calculated using EpiInfo 6.0 (USD, Inc, Stone Mountain, Ga) and EXACT 3.0.19 A forward, stepwise, multivariable logistic regression analysis was performed using SAS 6.09 (SAS Institute, Inc, Cary, NC).
lates from 5 patients who were residents of other states but exposed in Connecticut were determined to be indistinguishable from the outbreak-associated subtype.

**PATIENT CHARACTERISTICS**

Of 21 Connecticut outbreak-related cases identified, 1 was classified as a “secondary case” and excluded. The 20 patients included in the case-control study reported onset of illness during May 29 to June 27 (Figure 1). The median age was 50 years (range, 2-87 years), patients lived in 5 of 8 Connecticut counties, and 15 (75%) were female. Eighteen patients reported bloody diarrhea, 9 were hospitalized, and 2 developed hemolytic uremic syndrome (Table 1).

In Illinois, 23 (82%) of 28 patients who were infected with the outbreak-associated subtype were interviewed. Of the 5 patients not interviewed, 1 refused, 2 were not available for interviews, and 2 did not meet the case definition: 1 was determined to be a secondary case, and 1 was a nonresident. The 23 patients included in the case-control study reported onset of illness during May 28 to June 14 (Figure 1). The median age of patients was 35 years (range, 2-79 years), and 18 (78%) were female. Twenty-two patients reported bloody stools, 12 were hospitalized, and 1 developed hemolytic-uremic syndrome (Table 1).

Five persons were reported with E coli O157:H7 infections in 4 other states: Indiana, Massachusetts, New York, and North Carolina; all had a history of travel to Connecticut during their estimated exposure periods, and all were infected with the outbreak-associated strain.

**CASE-CONTROL STUDIES**

In Connecticut, 20 patients and 35 controls were interviewed for the case-control study. Only 1 exposure to “other types of lettuce” (defined as lettuce other than iceberg or romaine) was associated with illness. When analyzed by specific type of lettuce (red leaf, green leaf, mixed greens, or mesclun lettuce), only the association of illness with “mesclun lettuce” or “mixed-greens lettuce” categories was statistically significant. Follow-up calls were made to restaurants identified by patients and controls to determine which specific type(s) of lettuce was used in their mixed-greens salads. If component lettuces could not be determined, the salad was classified as “unspecified mixed greens.” After categorization and reanalysis, illness continued to be associated with consumption of mesclun lettuce (MOR, undefined; 95% confidence interval [CI], 3.4 to ∞; P < .001) and unspecified mixed greens (MOR, 14.0; 95% CI, 1.7-571.8; P = .006) (Table 2). Although the mixed-greens variable was significantly associated with illness, 6 case-patients (75%) who reported eating mixed greens also reported eating mesclun lettuce.

In Illinois, 23 case-patients and 46 controls were interviewed for the case-control study. Illness was associated with consumption of romaine and red leaf lettuce in the 7 days before onset of illness (Table 3). In multivariable logistic regression analysis, only red leaf lettuce remained associated with illness. To distinguish between small red leaves of lettuce included in mesclun lettuce and red-leaf bunch lettuce, 19 Illinois patients and 26 controls who reported lettuce consumption in the ini-

| Table 1. Patient Characteristics of Outbreak-Associated Escherichia coli O157:H7 Infections |
|----------------------------------|-----|-----|
|                                  | Connecticut | Illinois |
| Female                           | 15/20 (75)   | 18/23 (78) |
| Median age (range), y            | 50 (2-87)    | 35 (2-79)  |
| Bloody diarrhea                  | 18/20 (90)   | 22/23 (96) |
| Vomiting                         | 5/10 (50)    | 11/23 (48) |
| Fever                            | 6/10 (60)    | 8/23 (35)  |
| Hospitalized                     | 9/20 (45)    | 12/23 (52) |
| Hemolytic uremic syndrome        | 2/20 (10)    | 1/23 (4)   |

Figure 1. Outbreak-associated Escherichia coli O157:H7 infections in Connecticut and Illinois, May to June 1996.
tional case-control study were reinterviewed with a questionnaire focusing on lettuce exposure during the same period as the first questionnaire. This second study revealed an association between illness and consumption of mesclun lettuce (MOR, undefined; 95% CI, 1.4 to $^{*}$; $P = .009$) and green leaf lettuce (MOR, undefined; 95% CI, 1.1 to $^{*}$; $P = .02$). Red-leaf bunch lettuce was not significantly associated with illness (Table 3).

LETTUCE TRACE-BACK INVESTIGATION

In Connecticut, 6 (54%) of 11 patients with mesclun lettuce exposure were unable to recall the time and location of mesclun lettuce consumption. Five patients and 3 patient visitors to the state had a clear recall of eating mesclun lettuce at a total of 5 restaurants. One distributor supplied mesclun lettuce to all 5 restaurants during the patients’ exposure periods. One producer, company A, provided all of the mesclun lettuce sold by this distributor during May and June. Implicated lettuce from company A was delivered to retail outlets during May 24 to 31 (Figure 2).

Ten patients in Illinois reported having eaten mesclun lettuce during the week before onset of illness; 8 of these named a source that could be confirmed as having sold the lettuce during this period. Five patients had purchased mesclun lettuce from 4 grocery stores, and 3 patients had eaten mesclun lettuce at a restaurant. The 4 grocery stores represented 3 grocery store chains. Each of the 3 grocery store chains and 2 of the 3 restaurants had purchased mesclun lettuce from 1 distributor; this distributor was the source of the mesclun lettuce eaten by 7 of 8 patients. During May and June, the distributor had purchased mesclun lettuce from 2 different grower shippers, 1 of which was company A, the producer implicated in the Connecticut outbreak (Figure 2).

FIELD INVESTIGATION

Company A was inspected in July 1996 and found to be a small grower–producer of mesclun lettuce, shipping an average of 300 1.35-kg boxes per week. Baby lettuces and mustards (baby greens) were grown in 2 separate field locations and transported to a primary facility for processing.

One of the fields and the processing facility for the farm were located adjacent to a small beef cattle operation. Cattle lots were separated from the lettuce field by a dirt track. Free-range chickens had access to both cattle and lettuce fields.
The owner of company A denied using cow manure as fertilizer but reported that composted chicken manure may have been used on some fields in March. No field treatment records were available. Two wells were located on the property; well water was used for both cattle husbandry and lettuce culture and processing. Water from 1 well was used to flood irrigate a cattle pasture and to spray irrigate the lettuce fields. Spigots in the pasture were immersed in pools of water, some of which contained cow manure. Air bubbles were noted rising continuously from the spigots. The second well was located between the processing facility and cattle pens. The well supplied water for both lettuce processing and cattle water troughs.

One portable toilet was provided for approximately 10 employees. No hand-washing facilities were available. Gloves were not worn during lettuce processing. The lettuce-processing facility consisted of a large, 3-sided shed, with a smaller enclosed office and walk-in cooler behind the primary processing area. Baby greens were harvested, placed in reused boxes, and delivered to the facility in unrefrigerated trucks. Baby greens were sorted, immersed in a wash tank, manually agitated, then scooped out of the tank in reused plastic baskets, and spun in a centrifuge to remove excess water. In the rear of the shed, heads of frisée and radicchio lettuce were chopped on a plastic cutting board alongside visibly soiled boxes from the field. On the days of inspection, the chopped produce was rinsed in brown, murky water in a second small tank, then added to the baby greens to form the finished mesclun lettuce. Mesclun lettuce was packed into plastic-lined cardboard boxes and placed in the cooler. Company A transported the lettuce via refrigerated trucks to an air-freight facility where it was shipped overnight to customers.

The primary wash tank was acquired May 10, 1996, from another producer. The tank was filled with unchlorinated well water, which was recirculated through a polyvinyl chloride (PVC) pipe system to a set of canister filters before reentering the wash tank. On May 30, 1996, the filtration system broke and the recirculating system failed. From May 30 until the July inspection, the tank was reportedly filled with well water that was changed 3 times a day.

**ENVIRONMENTAL INVESTIGATION**

No *E coli* O157:H7 was isolated from the following samples: wash tank filters, wash water from both wash tanks, water from PVC pipes, finished mesclun lettuce, swabs from the cutting board and knife, well water, water from a cattle trough, water sampled from the cattle pasture, and cow or chicken manure.

Analysis of six 100-mL well-water samples yielded no *E coli*. A water sample from a lettuce wash tank yielded *E coli* (not O157:H7) MPN of 41 per 100 mL. Four water samples were obtained from PVC pipe in the recirculating water system, with a median *E coli* MPN of 165 per 100 mL (range, 39-816/100 mL). Frisée and radicchio lettuces were individual components of this mesclun lettuce formulation. Frisée lettuce sampled before processing yielded no *E coli*. Radicchio sampled before processing yielded *E coli* MPN 7 per100 g. Two samples of finished mesclun lettuce yielded *E coli* MPN of 45 and 97 per 100 g.

**LETTUCE TRACE-FORWARD INVESTIGATION**

Four states were identified during the trace-forward investigation as having directly received lettuce from the implicated producer during May 24 to 31: California, Florida, Illinois, and New York. In September 1996, health department personnel in California, Florida, and New York were asked to identify isolates collected during mid-May through June in each state and to perform PFGE analysis of these isolates along with an isolate collected from the multistate *E coli* O157:H7 outbreak. In California, personnel tested 40 of 42 *E coli* O157:H7 isolates collected during the outbreak period; none matched the outbreak-associated subtype. Public health personnel in Florida reported that no isolates were available for analysis. In New York, 7 (44%) of 16 *E coli* O157:H7 isolates received during mid-May through June were discovered to have PFGE patterns indistinguishable from the outbreak-associated subtype. The local distributor identified during the trace-back investigation from Connecticut was discovered to have supplied mesclun lettuce from company A to customers in both Connecticut and New York during May and June 1996.

This is the first reported multistate outbreak of *E coli* O157:H7 infections associated with consumption of lettuce. This outbreak resulted in infection and illness of at least 61 persons, with 21 hospitalizations, and 3 cases of hemolytic-uremic syndrome. This report highlights emerging trends in foodborne disease epidemiology: the increasingly important role of molecular subtyping of bacterial isolates and the emergence of produce as a vehicle for foodborne pathogens.

In previous foodborne disease outbreak investigations, molecular subtyping has been an important tool to help distinguish increases in sporadic disease from outbreaks, link widely dispersed cases to a common...
source, and improve the precision of case definitions. Comparison of different strains of *E coli* O157:H7 from a variety of locations can help identify sources of contamination and, in cases of ongoing contamination, prevent additional infections. To facilitate these kinds of comparisons, the Centers for Disease Control and Prevention has recently established a National Network of Molecular Subtyping (PulseNet). State public health laboratories are provided with standardized laboratory procedures and electronic links to share data among those with access to the network. As more states participate, this network will assume greater importance as a key element of the national strategic plan to improve food safety.

Molecular subtyping Provided information critical to this epidemiological investigation, effectively linking geographically separate but simultaneous outbreaks. In case-control studies in both states, the use of molecular subtyping led to greater precision in the selection of cases associated with the outbreak, and the discovery of a lettuce supplier common to both states led to a second case-control study in Illinois.

The potential for recall bias must be examined in regard to the second Illinois investigation, which was conducted 8 weeks after patient exposure. Prior to this study, there had been local media coverage of the outbreaks in both Chicago and Connecticut. In Chicago, illness was reported associated with red leaf lettuce. In Connecticut, local coverage focused on the association of illness with mesclun lettuce. It is unlikely that Chicago-area residents learned of the Connecticut study results as there was no national media coverage at the time. Chicago residents would predominantly have been aware of the association of illness with red leaf lettuce. In the second case-control study in Illinois, illness was significantly associated with mesclun, not red leaf lettuce, suggesting a lack of recall bias induced by these media reports.

In Connecticut and Illinois, mesclun lettuce was purchased by patients at multiple retail outlets, suggesting a central point of contamination prior to distribution. Trace-back investigations implicated lettuce produced and shipped by 1 company during May 24 to 31. Although *E coli* O157:H7 has been linked to lettuce in other outbreaks, this investigation expanded our knowledge of ways in which contamination may potentially occur during lettuce production.

Our field investigation revealed that the culture and processing practices of company A were subject to multiple potential sources of contamination: a neighboring cattle ranch, free-range chickens with access to both cattle and lettuce-growing areas, recirculation of wash water through a used filter, and PVC pipes containing thick, black organic material. Well water was distributed through pipes that supplied both cattle pastures and lettuce fields, cattle water troughs, and the lettuce-processing shed. There were no hand-washing facilities for workers and no hygienic controls during processing. The processing shed was open to the environment, with no mechanism to exclude dust, insects, birds, or rodents. Although *E coli* O157:H7 was not isolated from the lettuce in July, the presence of *E coli* bacteria on the finished lettuce product and in the wash water indicated the presence of fecal contamination from animals or humans.

Although the mechanism of inoculation with *E coli* O157:H7 is unknown, wash water was the most likely source of lettuce contamination. Until May 30, recirculated wash water passed from the washtub through PVC pipes and canister filters. After May 30, the washtub functioned as a static tank wash, and the water was reportedly changed 3 times a day. The last shipment of implicated lettuce occurred on May 31, the morning after lettuce-washing practices changed.

The trace-forward investigation from company A identified mesclun customers in 4 states. This led to the retrospective identification of 7 patients in New York infected with the outbreak-associated strain; the number of infections associated with consumption of mesclun lettuce was not discovered. During the same period, California received implicated produce, yet no outbreak-associated cases were detected. The reason for this lack of outbreak-associated cases in California is unknown. There may have been differences in case detection among states, or customers in Connecticut, New York, and Illinois may have received mesclun lettuce with a greater amount of *E coli* O157:H7 contamination and thus have been more likely to become infected after consumption of a mesclun salad.

As a natural, uncooked food product, lettuce has the potential to become contaminated with pathogenic microorganisms and transmit them to consumers. Ideally, all lettuce should be washed before consumption, but, because washing reduces but does not eliminate bacterial contamination, production and processing methods need to be designed to minimize the number of pathogenic microorganisms present in finished lettuce. Timely distribution with careful temperature control will help to reduce bacterial growth after harvest.

It is likely that the magnitude of this outbreak of *E coli* O157:H7 infections was greater than the 61 cases detected. As of June 1996, *E coli* O157:H7 was a reportable disease in 42 states, including Connecticut and Illinois. However, because most infected persons do not submit stool cultures and *E coli* O157:H7 is not routinely cultured in submitted stool specimens, there is a problem with underdiagnosis in the United States.

Outbreaks of enteric illness associated with raw produce items are likely to continue. Some types of raw produce, such as lettuce, are difficult to disinfect due to irregular surface features, fragile tissue structure, and the potential for internal contamination. The US food supply is increasingly dependent on food produced in large quantities in central locations. If contamination does occur during production, the food may be widely distributed and has the potential to cause illness in disparate geographic areas. Early detection and control of outbreaks may be enhanced through improved laboratory surveillance for *E coli* O157:H7 infections nationwide. Investigations of outbreaks are facilitated by PFGE subtyping, especially in those situations where a vehicle is on more than 1 geographic area is involved.
REFERENCES


