

An Outbreak of *Escherichia coli* O157:H7 Infections Associated with Leaf Lettuce Consumption

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In July 1995, 40 Montana residents were identified with laboratory-confirmed *Escherichia coli* O157:H7 infection; 52 residents had bloody diarrhea without laboratory confirmation. The median age of those with laboratory-confirmed cases was 42 years (range, 4–86); 58% were female. Thirteen patients were hospitalized, and 1 developed hemolytic-uremic syndrome. A case-control study showed that 19 (70%) of 27 patients but only 8 (17%) of 46 controls reported eating purchased (not home-grown) leaf lettuce before illness (matched odds ratio, 25.3; 95% confidence interval, 3.9–1065.6). Pulsed-field gel electrophoresis identified a common strain among 22 of 23 isolates tested. Implicated lettuce was traced to two sources: a local Montana farm and six farms in Washington State that shipped under the same label. This outbreak highlights the increasing importance of fresh produce as a vehicle in foodborne illness. Sanitary growing and handling procedures are necessary to prevent these infections.

Escherichia coli O157:H7 has been recognized as a major cause of hemorrhagic colitis and the hemolytic-uremic syndrome (HUS) in the United States [1]. Although most outbreaks of *E. coli* O157:H7 infection have been linked to foods of bovine origin, such as undercooked ground beef and dairy products [2], other food items of non-bovine origin, such as water, vegetables, cantaloupe, and apple cider, have been increasingly associated with infection [3–7]. From 1982 to 1996, 68% of reported outbreaks of *E. coli* O157:H7 infection had a food or beverage vehicle determined; from 1993 to 1996, 27% of these outbreaks were associated with food items of non-bovine origin, compared with 13% of outbreaks from 1982 to 1992 (CDC, unpublished data).

In July 1995, >70 persons in western Montana developed bloody diarrhea and abdominal cramps. Patients' stool cultures yielded *E. coli* O157:H7. In the previous year, a total of 8 persons with *E. coli* O157:H7 infection were reported from the same area, and only 1 case was reported in July. We report here the results of the epidemiologic investigation to determine the source(s) of the infection in these patients.

Materials and Methods

Case ascertainment. A case of *E. coli* O157:H7 infection was defined as diarrhea and abdominal cramps with laboratory evidence of *E. coli* O157:H7 infection (either a stool culture yielding the organism or an IgG antibody titer to the O157 lipopolysaccharide [LPS] of >80 [8]) occurring during July 1995 in a person residing in or near the Missoula and Bitterroot Valleys of western Montana and extending northward to Whitefish. We defined a possible case of *E. coli* O157:H7 infection as bloody diarrhea without laboratory evidence of *E. coli* O157:H7 infection occurring during July 1995 in a person residing in or near the Missoula and Bitterroot Valleys.

Initial cases were reported to the Missoula City-County Health Department on 15 July 1995 by a local hospital laboratory. To identify additional patients, we issued a press release on 19 July informing the public about the outbreak and requesting that persons with diarrhea and abdominal cramps contact the county health department. In addition, we contacted counties within Montana and the neighboring state health departments of Idaho, Wyoming, and Washington and asked them to notify us of recent cases of *E. coli* O157:H7 infection. Area health care providers were contacted by mail and requested to submit stool samples from symptomatic patients for culture of enteric pathogens, including *E. coli* O157:H7, and the area's two clinical laboratories were asked to culture all stools for *E. coli* O157:H7. Serum specimens were collected from patients with diarrhea and abdominal cramps for whom a cause had not been established.

Case-control study. The initial 28 patients identified with *E. coli* O157:H7 infection who were the first to become ill in their families were included in the case-control study. Secondary cases, defined as a second case of *E. coli* O157:H7 infection within a household occurring 1–5 days after an initial case, and 1 patient with an *E. coli* O157:H7 strain that did not match the outbreak strain by pulsed-field gel electrophoresis (PFGE), were excluded from the case-control study. Between 28 and 29 July, 2 age- and

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Informed consent was obtained from all study participants.

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telephone exchange—matched controls for each case were interviewed. Children <5 years old were matched within 1 year, children 5–9 years within 3 years, persons 10–19 years within 5 years, persons 20–59 years within 10 years, and those >60 years old within 15 years. Eligible controls had no history of diarrheal illness, abdominal cramps, nausea, vomiting, or fever during July and were living in or near the Missoula and Bitterroot Valleys during the time of the outbreak.

A questionnaire was administered by telephone by trained interviewers to patients and controls or their parents asking about illness, food consumption, shopping and food handling practices, and exposures that have been associated with past *E. coli* O157:H7 outbreaks. Patients were asked about the 5-day period before the illness began. Controls were asked about the same 5-day period in the week before the interview.

Laboratory investigation. Stool and serum specimens were collected from patients with illnesses consistent with *E. coli* O157:H7 infection. Stool specimens were plated onto sorbitol-MacConkey agar [9]. Sorbitol-negative colonies selected from sorbitol-MacConkey agar were identified biochemically and serotyped [10]. Isolates identified as *E. coli* O157:H7 were subtyped by PFGE [11]. *E. coli* O157:H7 isolates were tested by polymerase chain reaction for the presence of gene sequences encoding Shiga toxins 1 and 2 [12, 13]. Serum samples were examined for antibodies to *E. coli* O157 LPS by ELISA [8, 14].

Retail and field leaf lettuce samples, compost, irrigation water, and manure samples were sent to the US Food and Drug Administration district laboratory in Bothell, Washington, for culture by previously described methods [15].

Sheep and cow fecal samples were cultured for *E. coli* O157:H7 at Washington State University by previously described methods [16]. Irrigation water samples were divided into 30-mL aliquots, and each aliquot was combined with 30 mL of a double-strength concentrate of tryptic soy broth containing vancomycin (40 µg/mL) and cefixime (50 ng/mL), mixed, and incubated for 16–24 h at 45°C. The samples were then cultured by the same methods used for fecal samples.

Traceback. Since most patients reported purchasing at area grocery stores the leaf lettuce they consumed, traceback was initiated at the retail level. We asked grocery store produce managers in two western Montana counties to provide invoices for leaf lettuce purchased from 1 to 16 July 1995, and we questioned them about produce delivery, handling practices, and sales during the first 2 weeks in July. Traceback efforts were then extended to distributors, shippers, and growers of leaf lettuce.

Environmental investigation. On 21 July 1995, we began investigation of the implicated local produce farm and area grocery stores. We examined leaf lettuce growing, harvesting, and handling practices at the farm; delivery and distribution practices from farm to retail market; and leaf lettuce handling procedures within the retail stores.

We collected leaf lettuce samples from the retail market and the local farm's fields. Samples of irrigation water, manure, compost, and cow and sheep feces from the farm and nearby fields were also collected.

Statistical analysis. Univariate matched odds ratios (MORs) were calculated by use of maximum likelihood estimates and exact 95% confidence intervals (CIs) by Fisher's exact test [17]. Variables significantly associated with illness by univariate analysis

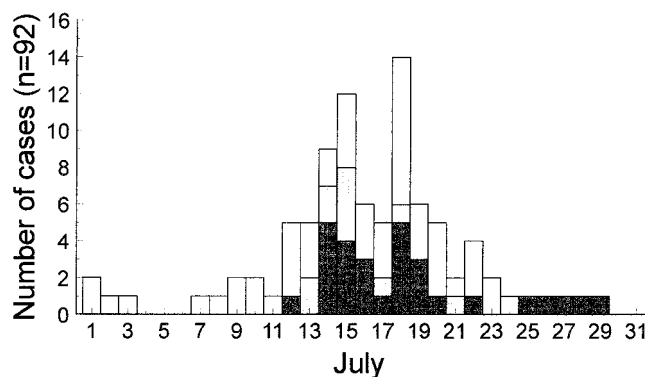


Figure 1. Possible and laboratory-confirmed cases of *E. coli* O157:H7 infection, by date of onset, western Montana, July 1995. Open bar, possible case; light shading, serologically confirmed case; dark shading, culture-confirmed case.

were entered into a forward stepwise multiple logistic regression model [18].

Results

Patient characteristics. We identified 40 laboratory-confirmed cases of *E. coli* O157:H7 infection in western Montana with symptom onset between 12 and 29 July 1995 (figure 1). The median age of patients was 42 years (range, 4–86); 58% were female. Symptoms included abdominal cramping (100%), diarrhea (100%), bloody diarrhea (87%), nausea (78%), vomiting (54%), and fever (35%). Thirteen persons were hospitalized; a 6-year-old child developed HUS. There were no deaths.

Among other persons reported to the health department, we identified 52 possible cases of *E. coli* O157:H7 infection with onset of bloody diarrhea during July 1995 (figure 1). Of these, 42 patients (81%) also reported abdominal cramps. The age distribution and proportion of females among the possible cases were similar to that of laboratory-confirmed cases.

Case-control study. Twenty-eight patients and 56 matched controls were included in the case-control study (table 1). Consumption of any lettuce was not associated with illness. However, consumption of leaf lettuce (red leaf, green leaf, bibb, or romaine) in the 5 days before illness onset was associated with infection (MOR = 4.0; CI = 1.5–12.7; $P < .01$). Consumption of purchased leaf lettuce (excluding home-grown lettuce) was even more strongly associated with infection (MOR = 25.3; CI = 3.9–1065.6; $P < .001$). Nineteen (70%) of 27 patients compared with 8 (17%) of 46 controls reported eating purchased leaf lettuce. Individual types of purchased leaf lettuce were also significantly associated with illness; red leaf lettuce had the strongest association (MOR = 22.0; $P < .001$; table 2).

In univariate analysis, several other factors, including purchasing groceries at grocery store A and consumption of oranges or homemade orange juice and strawberries, were associated with *E. coli* O157:H7 infection. However, in a multivariate model that included purchasing groceries at grocery store A and

Table 1. Frequency of selected exposures among patients and controls in univariate analysis, western Montana, July 1995.

Exposure	No. of patients/ total (%)	No. of controls/ total (%)	Matched odds ratio (95% confidence interval)	P
Any lettuce	25/28 (89)	42/56 (75)	3.2 (0.7–30.8)	NS
Leaf lettuce	19/27 (70)	18/56 (32)	4.0 (1.5–12.7)	<.01
Purchased leaf lettuce	19/27 (70)	8/46 (17)	25.3 (3.9–1065.6)	<.001
Purchasing groceries at store A	19/28 (68)	14/55 (26)	20.8 (3.1–887.8)	<.001
Oranges or homemade orange juice	5/28 (18)	1/56 (2)	10.0 (1.1–473.0)	<.05
Strawberries	17/27 (63)	19/54 (35)	3.3 (1.0–11.9)	<.05
Hamburger meat	13/27 (48)	32/56 (57)	0.74 (0.2–2.1)	NS
Any ground beef	19/28 (68)	42/54 (78)	0.55 (0.2–1.8)	NS
Milk	25/28 (89)	48/56 (86)	1.4 (0.3–8.6)	NS
Well water	11/27 (41)	27/53 (51)	0.52 (0.1–1.8)	NS
Washing lettuce before consumption	28/28 (100)	51/56 (91)	— (0.6)*	NS
Drying lettuce before consumption	16/28 (57)	28/51 (55)	1.2 (0.4–3.8)	NS

NOTE. NS = not significant.

* Lower 95% confidence interval only. Matched odds ratios and upper confidence intervals are undefined because there were no pairs for which control was exposed and case was not.

consumption of purchased leaf lettuce, oranges or homemade orange juice, and strawberries, only consumption of purchased leaf lettuce remained independently associated with infection ($P < .05$). Hamburger meat, any ground beef (including hamburger meat), milk, well water, and lettuce handling practices in the home were not linked with illness.

Laboratory investigation. Stool cultures from 29 patients yielded *E. coli* O157:H7. Eight of these patients also underwent serologic testing and had elevated titers to O157 LPS. An additional 11 patients had only elevated titers. The median reciprocal IgG antibody titer to O157 LPS for the 19 serologically confirmed patients was 320 (range, 160–640), compared with 56 (range, 40–80) for the 15 laboratory control serum samples. Of 29 *E. coli* O157:H7 isolates, 23 were confirmed as O157:H7 and subtyped by PFGE. Six isolates had been inadvertently discarded. PFGE patterns from these isolates were compared with those of 2 isolates from western Montana

patients with *E. coli* O157:H7 infection in the 6 months before July 1995 and 1 isolate from Idaho and 3 from Washington State from symptomatic patients in July 1995. A common pattern (outbreak strain) was identified in 22 of 23 isolates from western Montana patients in July 1995. Neither the earlier isolates nor isolates submitted by neighboring states matched the outbreak strain (figure 2).

All 23 *E. coli* O157:H7 isolates tested produced both Shiga toxins 1 and 2 and were susceptible to chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, nalidixic acid, ampicillin, sulfisoxazole, streptomycin, kanamycin, gentamicin, ceftriaxone, and amoxicillin-clavulanic acid.

Traceback. Fifteen patients who recalled eating leaf lettuce were able to provide the names of six grocery stores where they purchased the lettuce in the week before becoming ill. These six stores had received shipments from three distributors who had obtained their lettuce from two shippers. One shipper

Table 2. Frequency of purchased leaf lettuce exposures among patients and controls in univariate analysis, western Montana, July 1995.

Exposure	No. of patients/ total (%)	No. of controls/ total (%)	Matched odds ratio (95% confidence interval)	P
Red leaf	11/27 (41)	1/55 (2)	22.0 (3.2–947.0)	<.001
Green leaf	10/26 (38)	7/48 (15)	6.8 (1.4–65.6)	<.01
Romaine	9/25 (36)	2/51 (4)	13.8 (1.9–611.3)	<.01
Bibb	2/25 (8)	1/53 (2)	— (0.2)*	NS

NOTE. NS = not significant.

* Lower 95% confidence interval only. Matched odds ratios and upper confidence intervals are undefined because there were no pairs for which control was exposed and case was not.

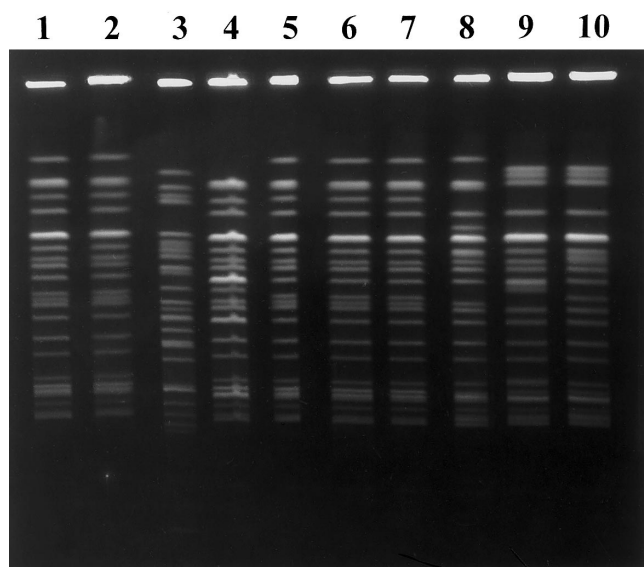


Figure 2. Pulsed-field gel electrophoresis patterns of *E. coli* O157:H7 isolates. Lanes 1, 2, 5–7: outbreak isolates. Lanes 3, 8–10: sporadic isolates not linked to outbreak (3, Montana; 8, Idaho; 9 and 10, Washington). Lane 4, unrelated food isolate recovered during same time period as outbreak.

had received leaf lettuce from a group of six farms located near each other in Washington State who shipped under the same label. Lettuce from these six farms was widely distributed in the northwestern United States (Washington, Idaho, Wyoming, and other regions in Montana) during July 1995. The other shipper was a small local produce grower who supplied leaf lettuce, herbs, and vegetables to area grocery stores and restaurants.

Environmental investigation. The local produce grower began harvesting leaf lettuce on 10 June, becoming fully operational on 20 June. Leaf lettuce (red leaf, green leaf, bibb, and romaine) was harvested almost daily, packed unwashed in cardboard boxes with 24 heads/box, and delivered three or four times weekly to area grocery stores and restaurants. Dairy composted manure, aged for 1 year, was used as fertilizer. The grower did not own cattle but did raise sheep, which were kept in a nearby pen. The farm obtained its irrigation water from a nearby pond supplied by several streams that passed through cattle fields. The grower, family members, and workers denied experiencing diarrhea during the harvest season.

None of the 37 samples of this grower's lettuce obtained from grocery stores and the field nor 6 samples of water, 4 samples of compost, 3 manure samples, or 58 sheep and cow fecal samples collected from the farm or adjacent fields yielded *E. coli* O157:H7.

Investigation of the six grocery stores where patients had reported purchasing lettuce did not reveal any areas that permitted contact between produce and raw meat. However, four (67%) of the six stores followed a leaf lettuce handling practice known as "crisping." Crisping consists of submerging leaf

lettuce heads in a basin of tepid water followed by refrigeration in the store's cooler to improve the lettuce's appearance. The basin water was changed infrequently, and numerous cartons and types of leaf lettuce were bathed in the same water. Iceberg lettuce did not undergo crisping. It either arrived at the store wrapped in plastic or was wrapped on the premises.

Discussion

To our knowledge, this is the first reported community outbreak of *E. coli* O157:H7 infections associated with consumption of lettuce. Eating purchased leaf lettuce was strongly associated with illness; 70% of patients in the case-control study reported consuming purchased leaf lettuce. Identification of produce that is usually eaten raw as a source of *E. coli* O157:H7 infections has important implications for the growing, handling, and preparation of produce.

Although the traceback investigation was not able to distinguish between the six closely linked farms in Washington and a local farm as the source of the implicated lettuce, we believe the latter to be a more likely source for two reasons: the few cases of *E. coli* O157:H7 infections reported in other states and regions that had received leaf lettuce from the six Washington farms and differences in PFGE patterns between western Montana isolates and those from other states in the same time period. These factors suggest that the contaminated leaf lettuce had a limited area of distribution. Although it is not known how contamination of leaf lettuce occurred, there are at least four possibilities. First, the farm fertilized its leaf lettuce with compost that contained manure obtained from a local dairy. Studies of cattle herds have shown that 2.8–3.3/1000 cattle carry *E. coli* O157:H7 [19], and *E. coli* O157:H7 has been shown to survive in cattle feces for up to 70 days [20]. Compost at this farm was reported to be aged for 1 year and reached temperatures of 40–60°C. However, if improperly aged compost was contaminated with *E. coli* O157:H7 and gained access to the fields, it could have directly contaminated the produce, as has occurred in the past [5]. Second, if infected cattle feces were present in the adjacent uphill pasture, these feces could contaminate either the water used for irrigating the fields (flood irrigation) or surface water runoff, which could then contaminate the lettuce. Third, since cattle had access to the streams above the pond used for irrigating the lettuce, their feces could have contaminated this water directly. Last, feces of other animal reservoirs of *E. coli* O157:H7, such as the sheep kept on the farm or deer, could also have contaminated irrigation water or the lettuce [21, 22].

Previous outbreaks of *E. coli* O157:H7 infections have been associated with foods of non-bovine origin. These investigations often found a link between the implicated food vehicle and cattle or cattle feces. Consumption of vegetables from a manured garden caused an *E. coli* O157:H7 infection in Maine. The same strain of *E. coli* O157:H7 was cultured from both the patient and manure from the garden [5]. In 1991, an outbreak of *E. coli* O157:H7 infections associated with consumption of

unpasteurized apple cider was attributed to use of apples collected from the ground that may have become contaminated by manure [7]. Handling of raw potatoes that were packed in peat that may have been contaminated with calf manure was linked to another outbreak of *E. coli* O157:H7 infections [4].

The tight clustering of cases in time and the lack of cases traced to restaurants or other retail markets supplied by the local grower suggests that the amount of lettuce originally contaminated was probably small and limited to one or two deliveries. The elevated odds ratios for different varieties of leaf lettuce suggests that either more than one type of lettuce was originally contaminated or that cross-contamination occurred among batches of lettuce. The latter possibility may have been facilitated by the practice of crisping, since numerous batches of leaf lettuce were processed in the same water. Since the infectious dose of *E. coli* O157:H7 is small (<1000 organisms [23]), one contaminated batch of lettuce in lukewarm water could produce a "broth" that could easily contaminate other heads of lettuce.

Although testing of lettuce, composted manure, water, and cattle feces did not yield *E. coli* O157:H7, these samples were collected ~2–3 weeks after the contaminated lettuce would have been harvested, distributed, and consumed. Lettuce is a biologically plausible vehicle because growth studies on lettuce have shown that bacterial populations of *E. coli* O157:H7 increased at 12°C and 21°C for up to 14 days [24]. Most retail stores display their produce at temperatures above 12°C.

After this outbreak, investigations of at least four other outbreaks of *E. coli* O157:H7 infections have implicated lettuce [25–28]. These outbreaks highlight the prominence of lettuce specifically and raw produce in general as potential vehicles in foodborne outbreaks. As demonstrated by the recent outbreak of >6000 cases of *E. coli* O157:H7 infections linked to radish sprouts in Japan [29], contaminated produce can have far-reaching consequences. Efforts to prevent these outbreaks must consider all elements of the complex path taken by fresh produce from farm to table. The risk of such outbreaks may be reduced by the use of uncontaminated irrigation water and efforts to protect produce from contact with cattle or cattle feces during cultivation and harvest by growers and by separating raw meat and produce during transportation and storage. Retail practices, such as lettuce crisping, which have the potential for cross-contamination should be avoided. It would be prudent to use chlorinated water during processing steps such as rinsing (and to carefully monitor chlorine levels). Recently, in consultation with state and federal health agencies, produce trade groups have published voluntary guidelines to minimize the risk of microbial contamination during fresh produce production and distribution [30]. Finally, consumers can reduce their risk of infection by continuing to avoid the simultaneous preparation and storage of raw meat and produce and by washing all produce carefully.

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