Multistate Outbreak of *Escherichia coli* O157:H7 Infection Associated with Consumption of Packaged Spinach, August–September 2006: The Wisconsin Investigation

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**Background.** *Escherichia coli* O157:H7 infection often causes hemorrhagic colitis and hemolytic uremic syndrome.

**Methods.** In 2006, the Wisconsin Division of Public Health and the Wisconsin State Laboratory of Hygiene, in cooperation with other local, state, and federal partners, investigated an outbreak of *E. coli* O157:H7 infection.

**Results.** In September 2006, the Wisconsin Division of Public Health and the Wisconsin State Laboratory of Hygiene were able to link geographically dispersed *E. coli* O157:H7 isolates recovered from the stool samples of ill persons, all of which had the same pulsed-field gel electrophoresis pattern (i.e., outbreak pattern). Investigators conducted a case-control study with control subjects (n = 86) matched to case patients (n = 49) by age, sex, and residential location. All case patients’ onset of illness occurred during the period from 20 August through 14 September 2006. Illness was associated with spinach consumption (matched odds ratio, 82.1; 95% confidence interval, 14.7 to >1000). Of the 49 case patients, 26 (53%) recalled eating brand A spinach. On multibrand analysis, only brand A was associated with illness (undefined matched odds ratio; 95% confidence interval, 6.8–∞). Wisconsin’s agriculture laboratory isolated *E. coli* O157:H7 with the outbreak pattern from spinach in 2 brand A packages, both produced on 15 August 2006.

**Conclusions.** The rapid multijurisdictional epidemiologic and laboratory response, including timely pulsed-field gel electrophoresis pattern analysis and PulseNet posting, facilitated prompt voluntary recall of brand A spinach.

The *Shiga toxin–producing strain Escherichia coli* O157: H7 is a major cause of foodborne illness [1, 2]. Cattle and other ruminants provide a reservoir for *E. coli* O157:H7 [3, 4]. Although outbreaks often have been associated with beef and dairy products [5–8], outbreaks attributable to fecal contamination of vegetables [9–11], to untreated water [12], and to cider and juices [13, 14] have been increasingly reported [7, 15]. *E. coli* O157:H7 infection often causes hemorrhagic colitis [2], although some cases of *E. coli* O157:H7 infection were asymptomatic or only caused watery diarrhea [2, 16]. Hemolytic uremic syndrome, a life-threatening complication, typically develops in 10%–20% of reported cases of *E. coli* O157:H7 infection and disproportionately affects children and older persons [2, 17].

On 5 September 2006, the Bureau of Communicable Diseases and Preparedness of the Wisconsin Division of Public Health received separate reports from local health departments regarding occurrences of clusters of laboratory-confirmed cases of *E. coli* O157:H7 infection in 3 noncontiguous counties: Manitowoc, Ozaukee, and Dane. The Manitowoc County cluster of cases involved
5 ill persons, 4 of whom visited an animal exhibition at a county fair.

On 7 September, the Wisconsin state epidemiologist for communicable diseases for the Wisconsin Division of Public Health was called by the director of the Blood Center of Southeastern Wisconsin regarding 5 adults in hospitals in 2 counties who received plasma exchanges during the prior 3 days to treat illnesses consistent with hemolytic uremic syndrome; 3 of the 5 adults already had confirmed cases of E. coli O157:H7 infection. To increase surveillance of E. coli O157:H7 infection, the Wisconsin Division of Public Health staff used the Wisconsin Health Alert Network and e-mail to notify local, regional, and tribal health departments; laboratories; infection control professionals; hospitals; emergency departments; and clinics of the suspected outbreak of E. coli O157:H7 infection.

During 6–8 September, molecular subtyping of 8 E. coli O157:H7 isolates was completed by staff at the Wisconsin State Laboratory of Hygiene by use of PFGE after DNA was digested with the restriction enzyme XbaI; 7 of these isolates had PFGE patterns that were indistinguishable from one another (hereafter referred to as the outbreak pattern), and the patterns were uploaded to PulseNet (the national molecular subtyping network for foodborne disease surveillance). It should be noted that, among the microbiologic tests completed soon after 8 September, the isolates recovered from the 4 Manitowoc County fairgoers had PFGE patterns that did not match the outbreak pattern, but an isolate recovered from the person who had not gone to the fair had a PFGE pattern that was indistinguishable from the outbreak pattern.

By 12 September, state laboratories and health departments in Oregon, Utah, and New Mexico reported to PulseNet that they had recovered E. coli O157:H7 isolates with the outbreak pattern, indicating a possible multistate outbreak. On 13 September, during discussions of the initial epidemiologic findings in Wisconsin and Oregon among staff of the Wisconsin Division of Public Health, the Oregon Department of Human Services, and the Centers for Disease Control and Prevention (CDC), it was revealed that multiple patients in each state who were infected with E. coli O157:H7 with the outbreak pattern had recently eaten spinach. On 14 September, the CDC issued a health alert that described the outbreak and the preliminary findings. That evening, the US Food and Drug Administration (FDA) issued an advisory that warned consumers not to eat bagged fresh spinach [18].

Case-control study. A case-control study was initiated on 8 September to identify the means of transmission of E. coli O157:H7 infection. We attempted to match 2 control subjects per case patient by age, sex, and geographic area by using a reverse telephone directory that identified residential neighbors. If no control subjects were identified, then control subjects were selected by identifying the telephone numbers of the respective case patients and progressively dialing telephone numbers 1 digit higher and lower. One control subject was selected per household. Control subjects were matched to case patients by age to within 5 years if the case patient was <10 years of age, to within 5 years if the case patient was >10 but ≤25 years of age, and to within 10 years if the case patient was ≥25 years of age. Consent was obtained from parents or guardians for interviews with children aged <18 years. Parents or guardians were interviewed if the case patient or control subject was ≤15 years old. A potential control subject was excluded if that control subject was ill with diarrhea or was vomiting during the 14 days before the matched case patient’s illness onset date.

Case patients and control subjects were interviewed during the
Outbreak of *Escherichia coli* O157:H7 Infection

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Figure 1. Epidemic curve of an outbreak of *Escherichia coli* O157:H7 infection among 49 case patients in Wisconsin, August–September 2006.

period 8–28 September. Personnel from the local health department and from the Wisconsin Division of Public Health interviewed case patients and control subjects using the Wisconsin questionnaire exclusively during the period 8–17 September. After 17 September, personnel from the local health department, the Wisconsin Division of Public Health, and the CDC interviewed case patients and control subjects using both the Wisconsin questionnaire and the spinach questionnaire. Case patients and control subjects initially interviewed only with the Wisconsin questionnaire were interviewed again with the spinach questionnaire.

Statistical analysis. Analyses were conducted by use of SAS, version 9.1 (SAS). Matched analyses using conditional logistic regression were conducted; matched exact ORs and 95% CIs were used to evaluate associations. Because many surveys only had affirmative responses indicated on the Wisconsin questionnaire, case patients and control subjects with missing responses to specific food items were assumed to have not had exposure.

We asked case patients and control subjects about their consumption of 4 specific brands of spinach products (brands A–D). A multivariable (i.e., multibrand) analysis that treated each brand as a separate variable was conducted to compare the effects of consuming any spinach from a particular brand. A sensitivity analysis was conducted to address classification problems if persons reported that they were uncertain whether they had consumed a particular brand.

Product testing. The Bureau of Laboratory Services of the Wisconsin Department of Agriculture, Trade, and Consumer Protection analyzed spinach from opened bags from which spinach had been consumed by case patients. The bags of spinach were collected by local health department personnel, packaged with a coolant, and sent by courier or commercial delivery to the Bureau of Laboratory Services for testing.

To culture and isolate *E. coli* O157:H7 from spinach samples, the staff at the Bureau of Laboratory Services used the FDA's preferred laboratory methods for microbiological analyses of food (i.e., the Bacteriological Analytical Manual) [20] and a detection system that uses PCR (BAX; DuPont Qualicon) [21]. A modification to these methods, using an elevated incubation temperature of 42°C, was also used. Isolation methods included immunomagnetic separation [22] and dilution plating. Tellurite-cefixime-sorbitol MacConkey agar and a chromogenic medium (CHROMagar O157; DRG) were used in both methods to suppress normal food flora. The staff at the Bureau of Laboratory Services delivered *E. coli* O157:H7 isolates cultured from each of the spinach samples to the Wisconsin State Laboratory of Hygiene for molecular subtyping by PFGE, using techniques identical to those used for analysis of clinical isolates.

Figure 2. Bar graph of the spinach consumption history of the 49 case patients infected with *Escherichia coli* O157:H7 in Wisconsin, August–September 2006, stratified by age group. Case patients ranged in age from 1 to 84 years.
**Shopper card.** We conducted a shopper card investigation to refine the data we were getting with regard to the products of concern. Case patients were asked for their shopper card number and whether they would permit the Wisconsin Division of Public Health to review their purchase history. A major grocery chain in Wisconsin (which includes multiple brands in their stores) provided a list of all purchases made with participants’ shopper card numbers during the 4 weeks before the respective illness onset. Purchase histories were compared with self-reported consumption.

**RESULTS**

**Outbreak investigation.** Our investigation included 49 Wisconsin residents (i.e., case patients) from 10 counties, with illnesses meeting the case definition. Among case patients, illness onset dates ranged from 20 August through 14 September (figure 1), and ages ranged from 1 to 84 years (figure 2). Of these 49 case patients, 35 (71%) were female, 24 (49%) were hospitalized, and 9 (18%) had hemolytic uremic syndrome. One case patient died of complications from hemolytic uremic syndrome. The most frequently reported signs and symptoms among the 49 case patients included diarrhea (47 patients [96%]), abdominal cramps (47 patients [96%]), bloody diarrhea (43 patients [88%]), fatigue (39 patients [80%]), watery diarrhea (31 patients [63%]), and chills (28 patients [57%]). Of these 49 case patients, 46 (94%) reported eating spinach, 45 (92%) reported eating fresh spinach during the 2 weeks prior to illness onset, and 31 (63%) reported eating fresh strawberries during the 2 weeks prior to illness onset. The time from illness onset to the PFGE report ranged from 6 to 23 days.

**Case-control study.** We interviewed 49 case patients and 86 control subjects for the case-control study. On bivariate analysis, spinach consumption was associated with illness (matched OR, 82.1; 95% CI, 14.7 to >1000). All other surveyed exposures (e.g., strawberries, other produce, and/or bovine products) were examined using matched conditional logistic regression; illness was not associated with these exposures, but cheese consumption appeared to have a protective effect (matched OR, 0.2; 95% CI, 0.1–0.7) (table 1). After controlling for spinach consumption, however, it was determined that cheese consumption did not have a protective effect. Consumption of brand A spinach was reported by 26 (53%) of 49 case patients, and 4 (5%) of 86 control subjects. In a multibrand analysis that included brands A–D, infection was associated with the consumption of brand A spinach (undefined matched OR; 95% CI, 6.8–∞) (table 2). The sensitivity analysis was consistent. In addition, when we examined the reporting of brand A spinach consumption among case patients, we noted that fewer patients aged ≥50 years recalled having consumed brand A spinach (figure 2).

Spinach preparation methods were examined. Of the 46 case patients who reported eating spinach, 10 (22%) reported washing prewashed spinach before consumption; of the 19 control subjects who reported eating spinach, 2 (11%) reported washing prewashed spinach before consumption. Cooking spinach at 71.1°C (160°F) for ≥15 s should kill contaminating *E. coli* [23]. Of the 46 case-patients and 19 control subjects who reported eating spinach, no case patients and 4 control subjects (21%) reported that they only ate cooked spinach.

**Product testing.** Eleven previously opened bags of spinach and 1 nonpackaged spinach specimen from case patients’ residences were received and tested at the Bureau of Laboratory Services. *E. coli* O157:H7 isolates with the outbreak pattern were recovered from spinach samples from 2 bags corresponding to 3 case patients (figure 3); 1 bag was delivered by courier...
The spinach samples tested from 2 of the 3 bags with product code P227A yielded O157:H7 isolates (table 3). These first 5 characters with the outbreak pattern had the same first 5 characters of the outbreak pattern, whereas no samples from the other 9 bags yielded E. coli O157:H7 isolates (table 3). One spinach sample from a bag with product code P227A tested negative for E. coli O157:H7, but that sample was from a bag that contained <25 g of decomposed spinach.

**Shopper card investigation.** Shopper card information identified the date of the purchase of spinach, the price paid, and the brand, but not the product code. Of the 27 case patients with available shopper card information, 17 (63%) purchased brand A spinach; of these 17 case patients, 2 (12%) had shopper card information that indicated a purchase of brand A spinach through self-reported consumption and shopper card purchase history (n = 14), self-reported consumption only (n = 12), or shopper card purchase history only (n = 3). This product was included in the voluntary recall initiated on 15 September on the basis of preliminary epidemiologic information. The Wisconsin investigation, having initially identified the outbreak and, concurrently with the Oregon Department of Health Services, having established the association with bagged fresh spinach, was an instrumental part of a national response to the outbreak that ultimately involved 205 laboratory-confirmed cases in the United States, which included 103 hospitalizations, 31 cases of hemolytic uremic syndrome, and 3 deaths [24]. CDC staff coordinated a broader national investigation, and Utah and New Mexico health department personnel conducted a similar but smaller case-control study [25]. An environmental investigation conducted by the FDA and the California Department of Health Services identified E. coli O157:H7 isolates with a PFGE pattern indistinguishable from the outbreak pattern in samples obtained from river water, cattle manure, and wild pig feces in and around a field used to grow brand A spinach with the P227A product code [24]. Beginning with Wisconsin’s recognition of this outbreak, the rapid response of the public health system around a field used to grow brand A spinach with the P227A product code [24]. Beginning with Wisconsin’s recognition of this outbreak, the rapid response of the public health system involved district personnel conducting a similar but smaller case-control study [25]. An environmental investigation conducted by the FDA and the California Department of Health Services identified E. coli O157:H7 isolates with a PFGE pattern indistinguishable from the outbreak pattern in samples obtained from river water, cattle manure, and wild pig feces in and around a field used to grow brand A spinach with the P227A product code [24].

**Table 3. Characteristics of the 12 spinach samples obtained from case patients’ households in Wisconsin, August–September, 2006.**

<table>
<thead>
<tr>
<th>Product code</th>
<th>Production day</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>P219A01</td>
<td>7 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>P227A</td>
<td>15 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>P227A02</td>
<td>15 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>P227A01</td>
<td>15 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>P228A03</td>
<td>16 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>P233A021</td>
<td>21 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>P236A02</td>
<td>21 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>P238B02</td>
<td>26 August</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>Missing</td>
<td>Unknown</td>
<td>Brand A baby spinach</td>
</tr>
<tr>
<td>No code</td>
<td>Unknown</td>
<td>Nonpackaged spinach</td>
</tr>
<tr>
<td>1237A5YM</td>
<td>25 August</td>
<td>Brand B spinach</td>
</tr>
</tbody>
</table>

a The last 2 characters were torn off this bag.
b Culture samples were positive for Escherichia coli O157:H7; isolates from these samples had PFGE patterns that were indistinguishable from the outbreak pattern.
and PulseNet posting) and the cooperation among many partners permitted FDA officials to quickly announce a broad national alert regarding fresh spinach and, within 15 days, to narrow the focus of environmental investigation to spinach that was processed by a single company and grown in 3 California counties.

Four notable factors contributed to Wisconsin’s success in rapidly identifying this outbreak. First, the rapid submission of samples was encouraged in Wisconsin by a preexisting agreement involving the Wisconsin Division of Public Health and the Wisconsin State Laboratory of Hygiene to allow fee-exempt testing of *E. coli* O157:H7 and other potential outbreak-related pathogens and by the use of a courier system to expedite delivery. Second, the staff at local health departments responded aggressively to the outbreak, and the state-wide use of the Wisconsin questionnaire facilitated active participation and rapid provision of data. Third, multimodal communication, which included the Wisconsin Health Alert Network, e-mail, press releases, and telephone calls, facilitated early detection and a coordinated response to the outbreak. Finally, the staff at the Wisconsin Division of Public Health could view PFGE patterns posted on PulseNet, and the Wisconsin Division of Public Health employed an epidemiologist trained in analyzing PFGE patterns. As patterns were uploaded, the staff at the Wisconsin Division of Public Health could immediately connect this information to the epidemiologic data from the emerging outbreak.

As in previous enteric disease outbreak investigations that involved the use of molecular subtyping [9, 26, 27], PFGE testing conducted by the staff at the Wisconsin State Laboratory of Hygiene was instrumental in linking geographically dispersed *E. coli* O157:H7 isolates recovered from the stool samples of ill persons, all of which had the same PFGE pattern (i.e., outbreak pattern), and also linked these human isolates to isolates from spinach [28]. Furthermore, early PFGE analysis distinguished nonoutbreak-related cases of *E. coli* O157:H7 infection, such as those associated with the Manitowoc County fair, from outbreak-related cases of infection. The selection criteria for PFGE used in the national case definition and in our report incorporated 1 enzyme matching the outbreak pattern (XbaI restriction pattern EXHX01.0124). However, PFGE testing at the Wisconsin State Laboratory of Hygiene included a second enzyme for testing isolates, to enhance discrimination of DNA patterns and increase cluster detection precision, but this procedure did not result in any exclusions of case patients.

Washing prewashed spinach before consumption might not decrease the risk of illness, because *E. coli* O157:H7 can be internalized by the spinach leaves [29]. In addition, *E. coli* O157: H7 can persist on vegetables for substantial periods after waterborne contamination [30], and available sanitation methods are not completely effective at removing organisms [31]. Evaluating the source of contamination requires examining the entire spinach growing and production process.

The accuracy of the information being recalled during an interview is important. As in a previous investigation [32], shopper card information helped confirm the accuracy of the information being recalled during an interview when public notification and recall of the presumed food source occurred before the majority of interviews occurred. Fewer persons ≥50 years of age recalled eating brand A spinach, but the concordance of recalled information with shopper card information was not associated with age. Further examination of the effects of age on the ability to recall brand information is warranted.

Our investigation had multiple limitations. If a case patient or control subject did not respond to a question from the Wisconsin questionnaire regarding the consumption of a certain food product, then that food product was assumed to have not been consumed. Nearly all data on spinach consumption habits (including brand of spinach purchased) were obtained after the initial FDA alert on 14 September and after media announcements, which likely facilitated a bias in the information being recalled by case patients and control subjects. Furthermore, brands other than brand A were infrequently mentioned by case patients and control subjects, which reduced the ability to detect whether another brand was involved. Also, a multivariable analysis comparing several risk factors was not possible because of the relatively small sample size, the difficulty of interviewees in recalling spinach-associated risk factors (such as preparation methods), and missing values.

Although this investigation proceeded rapidly, the recognition of and response to this outbreak possibly could have occurred earlier if clinicians, laboratory technicians, and public health practitioners systematically and rigorously employed the timeliest diagnostic, specimen-transport, and reporting methods. For the 49 case patients, the time from onset of illness to posting of a PFGE pattern on PulseNet was highly variable. Fortunately, for outbreak recognition and response, a sufficient number of individuals whose onset of illness occurred early in the outbreak were associated with the following sequence of events: they sought care quickly, their stool specimens were submitted for culture and were processed efficiently, and the isolates recovered from their stool specimens were expeditiously transferred from a clinical microbiology laboratory to the Wisconsin State Laboratory of Hygiene, where PFGE testing was done in a timely manner. Unfortunately, the decisions involved with testing and the time involved with testing and transport can vary widely between different detection systems and between different geographical regions. Unanticipated technical interruptions can create further delays. Detailed examination of these factors is needed to create greater efficiency in public health responses.

This and other outbreak investigations have demonstrated
that centralized, large-scale food production facilities with wide distribution networks can facilitate geographically widespread illnesses [33–35]. Because of continual risks for widespread illness, regulatory and public health system improvements are necessary to protect consumers and assure the safety of products in increasingly centralized food production and distribution systems. The FDA’s Lettuce Safety Initiative, launched in August 2006, should help decrease contamination of fresh lettuce and spinach [36].

Strengthening public health systems to prevent and control foodborne illnesses requires assuring timely reporting of suspect and confirmed cases of E. coli O157:H7 infection and other diseases. This also requires high indices of suspicion for foodborne illnesses, accurate diagnosis, and a commitment to maximize the information available through timely application of molecular subtyping and PulseNet posting of results and through timely responses to these data.

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