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Multistate outbreak of *Escherichia coli* O157:H7 infections associated with a national fast-food chain, 2006: a study incorporating epidemiological and food source traceback results

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SUMMARY

A multistate outbreak of *Escherichia coli* O157:H7 infections occurred in the USA in November–December 2006 in patrons of restaurant chain A. We identified 77 cases with chain A exposure in four states – Delaware, New Jersey, New York, and Pennsylvania. Fifty-one (66%) patients were hospitalized, and seven (9%) developed haemolytic uraemic syndrome; none died. In a matched analysis controlling for age in 31 cases and 55 controls, illness was associated with consumption of shredded iceberg lettuce [matched odds ratio (mOR) 8·0, 95% confidence interval (CI) 1·1–348·1] and shredded cheddar cheese (mOR 6·2, CI 1·7–33·7). Lettuce, an uncooked ingredient, was more commonly consumed (97% of patients) than cheddar cheese (84%) and a single source supplied all affected restaurants. A single source of cheese could not explain the regional distribution of outbreak cases. The outbreak highlights challenges in conducting rapid multistate investigations and the importance of incorporating epidemiological study results with other investigative findings.

Key words: Diarrhoea, *Escherichia coli* O157, lettuce, outbreak.

INTRODUCTION

Escherichia coli O157:H7, a major cause of haemorrhagic colitis and haemolytic uraemic syndrome (HUS), causes an estimated 73 000 illnesses and 61 deaths annually in the USA [1]. Of these illnesses, 85% are estimated to be caused by foodborne transmission [1]. Prevention of illnesses depends on identifying food vehicles and understanding the mechanisms of contamination in order to make contamination less likely in the future. Much of what is known about sources of foodborne pathogens, including *E. coli* O157:H7, has been learned from outbreak investigations [2]. Analytical epidemiological studies have been a powerful tool in this regard. However, results of epidemiological studies invariably need to be reconciled with other investigation findings, such as food supply information, or kitchen preparation and hygiene practices to ensure that the true outbreak source has been identified and control measures have been properly targeted.

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The most common foodborne sources of *E. coli* O157:H7 identified through outbreak investigations are foods of bovine origin, particularly ground beef [3–6] which accounted for 41% of *E. coli* O157:H7 outbreaks in the USA from 1982 to 2002 [2]. Produce items, the second most common food vehicle, caused 21% of foodborne *E. coli* O157:H7 outbreaks; dairy products caused 4% during that same time period [2]. Thirty outbreaks associated with leafy greens were reported to the Centers for Disease Control and Prevention (CDC) from 1973 to 2006 (CDC, unpublished data). With more centralized processing and widespread distribution of produce including leafy greens, the propensity for large multistate outbreaks to occur may have increased. The first reported multistate outbreak of *E. coli* O157:H7 infections associated with leafy greens consumption occurred in 1996 [7]. More recently, a large, well-publicized multistate outbreak of *E. coli* O157:H7 infections associated with spinach consumption occurred in September 2006 across 26 states resulting in 183 confirmed infections and three deaths [8].

Soon after the spinach outbreak, local health departments in New Jersey and New York independently identified local increases in *E. coli* O157:H7 infections in late November 2006. Both local investigations found that a majority of patients were patrons of chain A, a national Mexican-style fast-food restaurant chain. In New Jersey, because all of the initial patients consumed food from the same chain A location, the local health department presumed the outbreak to be localized and closed the restaurant on 30 November. Media coverage from the restaurant closure alerted the New York State Department of Health (NYSDH) of the New Jersey outbreak. On 3 December, NYSDH and New Jersey Department of Health and Senior Services communicated with each other and identified similar onset dates and a similarly high proportion of chain A exposure rates in their cases. On 4 December, CDC was notified and a coordinated investigation began. Within days, officials from Pennsylvania Department of Health and Delaware Department of Health and Social Services also reported illnesses in chain A patrons. Molecular subtyping data began to be uploaded into PulseNet, the national molecular subtyping network for foodborne disease surveillance, on 6 December. Cases in all four states had the same pulsed-field gel electrophoresis (PFGE) pattern suggesting they could have a common source. Affected restaurants were closed and a multistate investigation was initiated to confirm the association with the restaurant chain and to determine the likely food vehicle within the chain. Early in the investigation, chain A announced that green onions were the vehicle based on preliminary dipstick testing of green onions by a contracted private laboratory, but confirmatory testing of the same green onion samples in a United States Food and Drug Administration (FDA) laboratory did not yield *E. coli* O157. Public concern was heightened in the wake of the earlier spinach-associated outbreak, and the investigation took place against a background of considerable press and public interest. This report describes the results of the investigation and the methods used to determine lettuce as the likely vehicle.

METHODS

Surveillance and case finding

As part of routine surveillance, clinical laboratories that isolate *E. coli* O157 from human specimens submit isolates to state public health laboratories for PFGE subtyping. PFGE patterns are then uploaded to the central PulseNet database at CDC. Initial case finding was based on reports of *E. coli* O157:H7 infection reported through state public health departments in persons from Delaware, New Jersey, New York, or Pennsylvania with illness onset between 15 November and 14 December 2006. Once PFGE data became available and the epidemiological risk of chain A exposure was confirmed, we developed more focused case definitions. A confirmed case was defined as an infection with *E. coli* O157 with isolated PFGE pattern indistinguishable from the outbreak pattern (CDC PulseNet *Xba*I pattern no. EXHX01.1486) in a person who had illness onset between 15 November and 14 December 2006 and who had eaten at a chain A restaurant within 7 days prior to illness onset. A probable case was defined as non-culture evidence of *E. coli* O157 infection (i.e. Shiga toxin detection in stool, HUS) in a person who had illness onset between 15 November and 14 December 2006 and who had eaten at chain A restaurant within 7 days prior to illness onset in a state where a confirmed case had eaten at a chain A restaurant.

Case-control studies

Restaurant patronage

The parent company of chain A owns several fast-food chains in the geographic area of the outbreak.

We conducted a case-control study in order to confirm that illness was associated with eating at chain A restaurants only and not other restaurant chains or establishments.

This study was conducted in two states, New Jersey and Pennsylvania. Since PFGE information was not available for all cases at the time of the study, cases identified through initial case finding were enrolled. A case was defined as infection with *E. coli* O157 or non-culture evidence of *E. coli* O157 infection (i.e. Shiga toxin detection in stool, HUS) in persons residing in either participating state and with illness onset between 15 November and 7 December. Controls were well persons in the community matched to cases by neighborhood and identified via reverse phone directory method based on patient's address. The goal was to obtain two controls per case-patient. Case-patients and controls were interviewed using a questionnaire focused on restaurant exposures, particularly patronage of fast-food restaurants, in the 7 days prior to illness onset in the case. Study participants were enrolled from 7 to 10 December.

Food exposure at chain A

A second case-control study was simultaneously conducted in order to determine the food vehicle at chain A. Cases in this study were either confirmed or probable cases according to the outbreak case definitions for case finding. Controls were well dining companions identified by case-patients. Interviews focused on chain A menu items as listed on chain A's website, and included order modifications including any substitutions, additions, or withholding of certain ingredients (e.g. substituting beans for meat, adding cheese, withholding lettuce). Similar to the restaurant patronage study, study enrolment occurred from 7 to 10 December.

Traceback investigation

FDA traced the food vehicles implicated in the food exposure study back through the distribution chain to identify distributors, processing facilities, and growers. An environmental investigation of the farms was conducted by the California Food Emergency Response Team (CalFERT).

Laboratory investigation

E. coli O157 isolates from clinical specimens were subtyped by PFGE in state public health laboratories according to PulseNet protocols [9]; resulting patterns were submitted to the PulseNet database. The outbreak strain of *E. coli* O157:H7 was identified by PFGE using the endonuclease enzyme *Xba*I. Representative outbreak isolates were analysed in state public health and CDC laboratories to determine the Shiga toxin profile by polymerase chain reaction amplification of *stx* genes [10].

Food samples were tested for *E. coli* O157 from implicated restaurants and from leftover foods eaten at chain A by case-patients. Food testing was conducted by state public health laboratories, FDA, and CDC.

Statistical analysis

For both case-control studies, data were assembled using Microsoft Access 2003 (Microsoft Corp., USA), and analysed in SAS software version 9.1 (SAS Institute, USA). Data from both case-control studies were analysed using conditional logistic regression. For the food exposure study, menu items marked in the questionnaire were grouped by ingredients as indicated by recipes provided by the parent company of chain A. Primary analysis used exposures at the level of ingredients. Cases were matched to their meal companion controls. Potential confounding by age was addressed by including a term for age (<18 years *vs.* $\geqslant 18$ years) in bivariate analysis and multivariable models. Odds ratios (OR) and exact 95% confidence intervals (CI) were calculated.

RESULTS

Surveillance and case finding

During December 2006 to January 2007, *E. coli* O157:H7 isolates matching the outbreak PFGE pattern from 80 patients were uploaded into the PulseNet database. Of 78 patients interviewed, 70 (90%) reported eating food from a chain A restaurant within 7 days of illness onset. Collectively, 77 cases (70 confirmed, seven probable) residing in New Jersey (35), New York (22), Pennsylvania (16), Delaware (2), South Carolina (1), and Ontario, Canada (1) met the outbreak case definition. Dates of illness onset ranged from 20 November to 6 December 2006 with a peak of cases with illness onset on 24 November (Fig. 1). Fifty-three percent of patients were female. The median age was 18 years (range 4–61 years). Fifty-one patients (66%) were hospitalized; seven (9%) developed HUS, and none died.

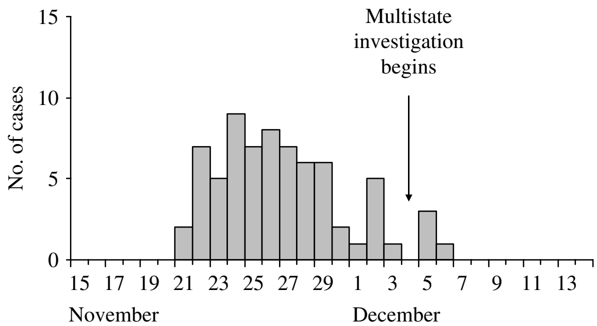

Fig. 1. Number of cases by illness onset date in a multistate outbreak of *Escherichia coli* O157:H7 infections, November–December 2006 ($n=70$).

Patients reported eating at a chain A restaurant in four northeastern states: Delaware, New Jersey, New York, and Pennsylvania (Fig. 2). Twenty-seven different locations of chain A restaurants were identified as a site of exposure in confirmed cases with a median of one case per restaurant (range 1–17).

Case-control studies

Restaurant patronage

A total of 35 cases of *E. coli* O157:H7 infection and 65 neighbourhood-matched controls were enrolled. Of eight major restaurant chains identified by study subjects, illness was only associated with eating at chain A in the 7 days prior to illness onset [29/34 (85%) cases, 2/64 (3%) controls, matched odds ratio (mOR) 72·6, 95% CI 12·9–∞). Of the 29 cases with reported exposure to chain A, 24 were subsequently found to have isolates with PFGE patterns matching the outbreak strain, three were probable cases with no PFGE data available, and two had variant PFGE patterns similar to the outbreak strain.

Food exposure at chain A

We enrolled 31 cases (30 confirmed and one probable) and 55 well meal companion controls. Recipes from chain A contained 35 different ingredients that were included in the analysis.

After controlling for age in a matched analysis, case-patients were more likely than controls to have consumed one of three food ingredients: shredded lettuce [30/31 (97%) cases, 40/55 (73%) controls; mOR 8·0, 95% CI 1·1–348·1], shredded cheddar cheese [26/31 (84%) cases, 25/55 (46%) controls; mOR 6·2, 95% CI 1·7–33·7], and taco shells [16/31 (52%) cases, 7/55 (13%) controls; mOR 16·6, 95% CI 2·4–719·0] (Table 1). Illness was not associated with any other ingredient or condiment.

All case-patients ate either lettuce or cheddar cheese; 25 (81%) consumed both ingredients. Five (16%) of 31 case-patients ate lettuce and not cheese, and only one (3%) ate cheddar cheese and not lettuce. Taco shells were consumed by a much lower proportion of case-patients than lettuce or cheddar cheese. Differentiating the association of illness with lettuce or cheese exposure through multivariable analysis was limited by the high level of concordance between lettuce and cheese. When controlling for age in a multivariable model containing all three exposures associated with illness, lettuce (OR 3·9, 95% CI 0·4–196·8) and cheddar cheese (OR 3·2, 95% CI 0·8–18·0) had similar adjusted associations, but lower than taco shells (OR 8·7, 95% CI 1·1–392·8). When the model was restricted to shredded lettuce and shredded cheddar cheese, the foods with highest exposure in cases, similar adjusted associations were found for lettuce (OR 6·0, 95% CI 0·7–304·1) and for cheddar cheese (OR 4·9, 95% CI 1·3–26·7).

Environmental and traceback investigation

FDA traceback investigations revealed that iceberg lettuce and cheddar cheese were each shredded before distribution to individual restaurants. The affected restaurants were supplied most ingredients through two chain A distribution centres. Two different manufacturers supplied shredded cheddar cheese made from pasteurized milk to implicated restaurants. One cheese shredder with one source of block cheese supplied the restaurants in Delaware, New Jersey, Pennsylvania, and New York City area through one distribution centre. A different cheese shredder with a different source of block cheese covered the upstate New York restaurants through a different distribution centre. For shredded lettuce, a single processor supplied both chain A distribution centres and therefore all implicated restaurants. Traceback of lettuce from four different restaurants in New Jersey, New York, and Pennsylvania converged to six fields in Huron, California in California's Central Valley [11]. Field investigations identified no risk factors for contamination, but fields had been ploughed at the time of investigation and were either planted with a cover crop or were fallow.

Laboratory investigations

The outbreak strain of *E. coli* O157:H7 was identified by PFGE to be CDC PulseNet pattern

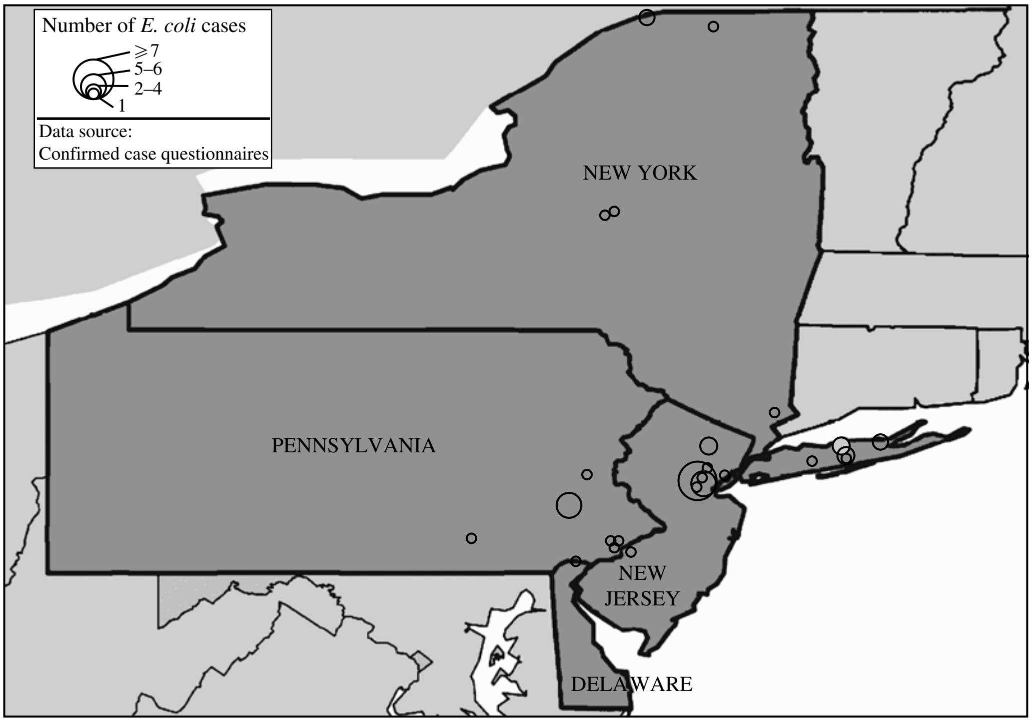

Fig. 2. Location of chain A restaurants associated with confirmed cases in a multistate outbreak of *Escherichia coli* O157:H7 infections, November–December 2006.

Table 1. *Ingredient exposure at chain A in cases and controls in a multistate outbreak of* Escherichia coli *O157:H7 infections, November–December 2006**

Exposure†	Cases exposure ($n=31$)	Controls exposure ($n=55$)	Exact OR	95% CI
Lettuce	**30 (97%)**	**40 (73%)**	**8·0**	**1·1–348·1**
Cheddar cheese	**26 (84%)**	**25 (46%)**	**6·2**	**1·7–33·7**
Ground beef	25 (81%)	34 (62%)	3·8	0·7–37·2
Tomato	17 (55%)	28 (51%)	1·6	0·5–6·1
Tortilla	17 (55%)	39 (71%)	0·3	0·1–1·3
Taco shell	**16 (52%)**	**7 (13%)**	**16·6**	**2·4–719**
Sour cream	16 (52%)	33 (60%)	0·8	0·2–2·4
Cheese sauce	12 (39%)	20 (36%)	1·2	0·2–5·8
Beans	9 (29%)	19 (35%)	1·0	0·3–3·6
Green onion	8 (26%)	11 (20%)	2·1	0·4–14·0
Flat bread	8 (26%)	10 (18%)	1·8	0·4–9·2
Chicken	8 (26%)	11 (20%)	0·6	0·1–2·1
Three-cheese blend	7 (23%)	20 (36%)	0·4	0·1–1·5

Bold values are statistically significant.
* Controlling for age (<18 yr *vs.* ⩾18 yr).
† Only exposures with >20% case exposure rate listed.

EXHX01.1486. Before this outbreak, this was a rare *E. coli* O157:H7 strain; as of 1 December 2006, this PFGE pattern accounted for only 20 (0·08%) of the 24 000 *E. coli* O157:H7 *Xba*I PFGE patterns in the PulseNet central database. During the outbreak period, 80 patterns of the outbreak strain PFGE pattern were uploaded into the database, accounting for 23% of all *E. coli* O157 patterns submitted to PulseNet nationally and 68% of patterns from affected states. The median number of days from onset date to uploading of the isolate PFGE pattern into PulseNet was 15 days (range 7–46 days). Laboratory analysis of the Shiga toxin profile found the outbreak strain produced Shiga toxin 2 (Stx2) only.

A total of 384 food samples were collected from restaurants, manufacturers, and case-patients including 39 samples of lettuce and 59 samples of cheese from implicated restaurants. A sample of white onions from a New York restaurant collected from an open bin in one implicated restaurant yielded *E. coli* O157. However, the PFGE pattern of the *E. coli* O157 isolated from white onions did not match the outbreak strain and did not match any isolates from human illness in the PulseNet database. No other food items tested yielded *E. coli* O157.

DISCUSSION

Considering the epidemiological results and the sources of implicated foods, this large multistate outbreak of *E. coli* O157 infections associated with a national Mexican-style fast-food chain in the USA was most likely caused by iceberg lettuce. This outbreak demonstrated the challenges of investigating a widespread outbreak, encompassing multiple health jurisdictions, involving menu items with overlapping ingredients, and the value of incorporating epidemiological and food source traceback information. Information on ingredient supply was crucial to differentiating closely linked exposures. Illness was statistically associated with eating shredded lettuce, shredded cheddar cheese, and taco shells. Multivariable analysis of lettuce, cheese, and taco shells showed only a statistically significant association with taco shells. However, the dry baked taco shells were consumed by a much lower proportion of patients than lettuce or cheese and were judged to be an unlikely source. Multivariable analysis of only lettuce and cheese showed a statistically significant association with cheese and not lettuce. This analysis was limited by the almost complete concordance of exposure between lettuce and cheese. Lettuce had the higher proportion of cases exposed, the higher odds ratios in both univariate and multivariable analysis, and more discordant pairs. Ultimately, traceback investigations of both lettuce and cheddar cheese helped determine that lettuce was the most likely source. Lettuce traced to a single processor with a distribution pattern consistent with the geographic distribution of the affected restaurants and the outbreak cases, and traceback of implicated lots of shredded lettuce converged on a small geographic area in California's Central Valley. There was no convergence in the tracing of shredded cheddar cheese to a single manufacturer or processor that could possibly explain the distribution of affected restaurants or all cases in the outbreak. Lettuce has historically been an important vehicle of *E. coli* O157 infections, increasing its biological and epidemiological plausibility.

Although 77 (70 confirmed, seven probable) cases were identified, the outbreak may have caused many more illnesses, considering the relative insensitivity of laboratory-based surveillance. Using a multiplier to account for this insensitivity derived from a study of sporadic cases, the number of illnesses due to this outbreak may be as high as 1500, although a multiplier based on sporadic cases may overestimate total outbreak cases if intense media coverage meant people were more likely to seek care and be cultured [1]. A large proportion of patients (70%) were hospitalized during the outbreak including seven (9%) patients who developed HUS. Although this proportion may reflect a surveillance bias towards more ill patients, it still represents a relatively large proportion of cases. This large proportion may be related to virulence factors of the organism. The outbreak strain was found to be Stx2-producing only. Some previous studies have demonstrated an association between increased severity of disease and strains producing Stx2 only compared to other strains [12].

To ensure a rapid public health response, we simultaneously conducted a case-control study to identify additional restaurant chains involved and a study to identify the food item at the chain identified during initial case interviews. Because most patients reported eating food from a chain A restaurant, we started the food item study prior to the results of the restaurant patronage study. However, we were concerned that the scope of the outbreak may have been larger than chain A because the parent company owned several other restaurant chains in the same geographic area which may have shared common suppliers. The main public health control measure applied during the outbreak was swift closure of implicated restaurants by local health authorities and chain A management, followed by disinfection and resupply of fresh ingredients. Because the restaurant patronage study did not find an association of illness with any other chains, public health action did not need to expand beyond actions taken at chain A restaurants.

This outbreak highlights the importance of interpreting analytical epidemiological studies within the context of the entire investigation, and conducting the investigation as rapidly as possible. Online availability of menus for chain A hastened the design and launch of the study. Analysis depended on ingredient information which was rapidly provided by chain A. Statistical analysis helped narrow the number of likely vehicles to lettuce and cheddar cheese. We ultimately integrated traceback information to show that all implicated restaurants could not have received cheddar cheese from the same source while lettuce for all implicated restaurants came from the same processor with traceback investigations converging to a small region in California's Central Valley. Although microbiological confirmation in the implicated food was not possible in this instance, the epidemiological and traceback information taken together are most consistent with lettuce as the likely source. The lesson learned is that one should not limit conclusions based on statistical associations alone, but rather continually re-evaluate the credibility of the evidence as new information is obtained. In this investigation, statistical modelling was severely limited by high concordance between lettuce and cheddar cheese. Ultimately, traceback data served as an important epidemiological tool to help determine the source of the outbreak. Improved traceability of food items can aid future outbreak investigations in a similar manner.

Our results are subject to several limitations. Chain A's announcement that green onions were the vehicle was based solely on preliminary dipstick testing; confirmatory testing by FDA did not yield *E. coli* O157. We were concerned that the announcement would lead to differential recall bias, with vigilant patients overreporting consumption of green onions compared to well control persons who may not have heard or remembered the announcement. By focusing questionnaires on menu items rather than individual ingredients, this recall bias was probably minimized. Few patients or controls consumed items with green onions. The food exposure study may have been affected by other types of recall bias. Several menu items were similar but had minor variations in ingredients.

Extensive testing of food products collected from implicated chain A restaurants failed to identify the outbreak strain. Due to high volume and turnover of fresh foods and their short shelf-life, it is likely that the original contaminated foods were consumed or discarded before the outbreak was detected. Extensive cleaning and disinfection in implicated restaurants may have played a role in preventing further infections through cross-contamination. Safe food preparation practices at restaurants are an important component of a comprehensive food safety programme. However, prevention of similar future outbreaks will depend on interrupting contamination earlier in the farm-to-table food production chain.

Outbreaks due to a perishable product with a short shelf-life are often over by the time the product is implicated and possibly recalled, so it is important to consider how outbreak detection and investigations might be accelerated. This outbreak was identified by state and local health departments based on an increase of *E. coli* O157 infections in their communities and reports of chain A restaurant patronage by ill persons. With a median of only one confirmed case per implicated restaurant, recognition of a multistate outbreak was dependent on communication between state and local health departments, state public health laboratories, and CDC, as well as use of PulseNet to link matching PFGE patterns. While PulseNet empowers states and federal officials to detect and investigate outbreaks sooner and with greater precision than in the past, successful investigation of these outbreaks depends on the capacity and communication of public health laboratories, epidemiologists, and environmental health specialists across multiple jurisdictions. Enhanced communication between health departments and CDC, more prompt reporting of PFGE patterns to PulseNet, and more rapid investigation methods may expedite detection and investigation of widespread outbreaks in the future.

Several mechanisms of contamination have been implicated in previous leafy green outbreaks, ranging from field-level contamination to cross-contamination during food handling [7, 13]. Given the multiple restaurants implicated, coincident cross-contamination within the kitchens by another food source, such as ground beef, is unlikely to explain this outbreak. Contamination most likely occurred before food arrived at the restaurants. Potential sources of contamination identified in past outbreaks associated with leafy greens have included use of contaminated water sprayed on the plants or used in processing, direct exposure to animal faeces, and use of improperly composted manure [7, 14]. Whatever the original source, a limited initial contamination may have been spread as the lettuces were mixed and shredded centrally before restaurant distribution. *E. coli* O157 readily internalizes into leafy green tissues, and while washing leafy greens can reduce surface bacterial contamination, it does not eliminate the risk [15]. Irradiation can reduce viable *E. coli* O157:H7 cells in leafy greens without affecting quality [16]. It has been approved by the FDA and may be particularly useful for centralized supply chains.

Although the precise mechanism of contamination was not identified for this outbreak, this outbreak, in the wake of other outbreaks of *E. coli* O157:H7 infections associated with leafy greens, greatly influenced industry practices and FDA policy. In early 2007, within a few months of this outbreak, the industry approved the California Leafy Green Products Handler Marketing Agreement under the supervision of the California Department of Food and Agriculture. This agreement made the industry responsible for self-regulation and enforcement of best production practices and use of an agreed upon traceback system. However, for these interventions to be successful, partnership in prevention needs to extend from farm to table, to minimize contamination of foods, especially those eaten raw.

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DECLARATION OF INTEREST

None.

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