



Department of Health and Human Services



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Subject: Multistate outbreak of *Escherichia coli* O157:H7 infections from lettuce consumed at a national fast food chain, Northeastern United States, November–December 2006

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BACKGROUND

On December 4, 2006, Centers for Disease Control and Prevention (CDC) officials were contacted by officials from the New Jersey State Health Department and from the New York State Health Department about outbreaks of *Escherichia coli* O157:H7 infections associated with a Mexican-style fast food restaurant chain in their states. By December 5, Pennsylvania and Delaware State Health Departments were also reporting illnesses associated with Chain A. On December 5, the CDC Outbreak-Net Team along with the involved states initiated a multistate case-control study to identify the source of these illnesses.

On December 6, 2006, Delaware submitted the first pulsed-field gel electrophoresis (PFGE) patterns related to the outbreak to PulseNet (the national molecular subtyping network for foodborne disease surveillance). That same day, the parent company of Chain A announced through the media that green onions were the source of the outbreak based on preliminary test results by a commercial laboratory which tested over 100 items from one restaurant. Chain A subsequently removed green onions from all restaurants within the chain nationwide.

METHODS AND MATERIALS

Case Finding

CDC contacted state health departments and notified public health officials of this outbreak on December 5. States identified outbreak cases primarily through routine case surveillance for *E. coli* O157 infection. Ill persons who contacted state health departments were instructed to seek medical care. CDC PulseNet also posted the outbreak PFGE pattern on the PulseNet webboard and asked states to report isolates. For surveillance purposes, a confirmed case was defined as infection with *E. coli* O157 with isolate PFGE pattern indistinguishable from the outbreak pattern in a person residing in the United States who had illness onset between November 15 and December 14, 2006 (or if illness onset date unknown, then *E. coli* O157 isolated between November 15 and December 14, 2006), and who had history of eating at Chain A within 14 days before illness. A probable case was defined as infection with *E. coli* O157 without PFGE results available or non-culture evidence of *E. coli* O157 infection (e.g. Shiga toxin detection, hemolytic uremic syndrome (HUS)) in a person who had illness onset between November 15 and December 14, 2006 (or if illness onset date unknown, then *E. coli* O157 isolated between November 15 and December 14, 2006), and who had history of eating at Chain A within 7 days in a state where a confirmed case had eaten at Chain A.

Case-control studies

Restaurant Patronage

The parent company of Chain A owns several other fast food chains in the geographic area of the outbreak. We conducted a case-control study to confirm that Chain A was associated and to determine if any other restaurants were associated with illness.

For efficiency, this study was conducted in two states, New Jersey and Pennsylvania. For this case-control study, a case was defined as infection of *E. coli* O157 with onset after November 15 in persons residing in New Jersey or Pennsylvania. Controls were well persons in the community matched to cases by neighborhood and obtained via a reverse phone directory method. The method required entering a patient's address into the website www.whitepages.com to obtain a list of neighbors to the case. Controls were then obtained by systematically calling those on the list until two controls obtained per case.

Questionnaires focused on restaurant exposures particularly exposure to fast food restaurants. Subjects were asked about patronage of fast food chains affiliated with the parent company of Chain A as well as other leading fast food chains.

Food Exposure at Chain A

A second case-control study was conducted to determine the food ingredients at Chain A that may have been the source of the outbreak. Cases in this study were either confirmed or probable cases as defined using the surveillance case definition. Controls were matched to cases based on exposure to the same Chain A location. The primary source of control recruitment was well dining companions of cases. A small number of controls were recruited from the community via reverse directory method. New Jersey was able to recruit controls from persons at a local university's student center who had eaten at the South Plainfield Chain A restaurant, the location

with the most cases associated. New York recruited controls from a list of well community patrons who had called local and state health departments at the beginning of the outbreak.

Questionnaires focused on Chain A menu items as listed on Chain A's website. In addition, questionnaires collected information from respondents on order modifications including substitutions, additions, and withholdings of individual ingredients (e.g. substitute beans for meat, hold lettuce, add cheese, etc).

Traceback Investigation

FDA conducted a traceback investigation of likely suspect vehicles.

Laboratory Investigation

E. coli O157 isolates from clinical specimens were serotyped and subtyped by PFGE at state public health laboratories.

Food samples were also tested for *E. coli* O157 from implicated restaurants and from leftover foods eaten by patients from Chain A. Food testing was conducted by state public health laboratories, Food and Drug Administration (FDA), and CDC. Although only 16% of the 384 food samples had food testing information reported, all of these reported testing by the Food Emergency Response Network (FERN) method.

Statistical Analysis

For both case-control studies, a database was created and maintained using Microsoft Access 2003. For the food item study, menu items marked in the questionnaire were grouped by ingredients of each of these items as indicated by recipes provided by the parent company of Chain A. Data for both studies were subsequently analyzed in SAS software version 9.1 (SAS Institute, Cary, NC). In the food item study, primary analysis was done based on exposure to the food ingredients. In the matched analyses for both studies, odds ratios and exact 95% confidence intervals (CI) to test for differences in risk between ill and well individuals were calculated via conditional logistic regression.

RESULTS

Case Finding

As of December 20, 2006, 77 cases (69 confirmed and 8 probable) were identified as meeting the surveillance case definition in five states: Delaware (2), New Jersey (36), New York (22), Pennsylvania (16), and South Carolina (1). (Figure 1) One additional case was reported from Ontario, Canada but was not included in the official case count because of residence outside of the United States. Patients reported Chain A exposure in only four states: Delaware, New Jersey, New York, and Pennsylvania. (Figure 2) Dates of illness onset ranged from November 20, 2006 to December 6, 2006 with a peak of cases with illness onset on November 24. (Figure 3) Fifty-five percent of patients were female. The median age of patients was 18 years with a range of 4-61 years. Fifty-five patients (72%) were hospitalized; 7 (9%) developed HUS, and none died.

Case-Control Studies

Restaurant Patronage

A total of 36 cases and 63 controls were enrolled. Among all of the restaurants studied, only Chain A had statistically significant association with illness. (mOR = 73.3, CI = 13.0 – Infinity) (Table 1).

Food Exposure at Chain A

Case-patients reported eating an average of 2.2 menu items compared to an average of 1.9 menu items reported by controls.

Among 68 patients interviewed, 64 (94%) had exposure to shredded lettuce. Cheddar cheese also had a high rate of exposure with 56 (82%) of patients reported exposure. Fifty-three patients (78%) reported ground beef consumption and fourteen patients (21%) reported green onion consumption.

Controlling for age in a matched analysis of 51 cases and 84 controls, cases were more likely than controls to be exposed to one of three food ingredients: shredded lettuce (OR = 5.5; 95% CI = 1.3-35.9), taco shells (OR = 3.9; 95% CI = 1.4-12.9), and shredded cheddar cheese (OR = 3.7, 95% CI = 1.5-10.6) (Table 2). A shredded blend of 3 cheeses, including cheddar cheese was not associated with illness (OR=0.4, 95% CI=0.2-1.1). Green onions were also not associated with illness (OR = 1.2, 95% CI = 0.3-4.2). Fiesta salsa and rice were found to have a protective effect (OR = 0.1, 95% CI = 0.0-0.6 and OR = 0.1, 95% CI = 0.0-0.9, respectively). When analysis was restricted to only those with matching well dining companions, there were 34 cases and 59 controls. Controlling for age again in a matched analysis, similar results were found among suspect vehicles: shredded lettuce (OR = 8.0; 95% CI = 1.1-345.7), taco shells (OR = 17.0; 95% CI = 2.5-737.5), and shredded cheddar cheese (OR = 6.7, 95% CI = 1.9-35.8).

Eight (16%) of the 51 cases from the matched analysis had exposure to lettuce and not cheese while only 2 (4%) had exposure to cheddar cheese and not lettuce. The collinearity of lettuce and cheddar cheese was further demonstrated by the fact that 40 cases (78%) had exposure to both lettuce and cheddar cheese. Only 1 case had no exposure to either lettuce or cheese. Several multivariate models were assessed to examine the relationship between lettuce or cheese consumption and illness. Comparisons between lettuce only versus neither lettuce nor cheddar cheese and cheddar cheese only versus neither resulted in similar associations when controlling for age (OR = 6.3, CI = 0.6-330 and OR = 7.8, CI = 0.3-677, respectively).

Subsequent traceback investigations revealed two separate distributors of cheddar cheese based on geography and thus two separate sources to implicated restaurants. To incorporate this into our analysis, we assumed that implicated restaurants in upstate New York received cheddar cheese from the Albany, New York distribution center while all other implicated restaurants received cheddar cheese from the Burlington, New Jersey distribution center. Cheddar cheese from the Burlington, New Jersey location had a case exposure rate of 78% with an odds ratio of 4.4 (CI=1.6-13.7). Cheddar cheese from the Albany, New York location had a case exposure rate of only 3.9% with an odds ratio of 1.8 (CI=0.02-172.1). Using individuals who had neither lettuce nor cheddar cheese as a reference, multivariate analysis that controlled for age and included terms for lettuce and cheddar cheese from the Burlington distributor found similar

statistical associations with illness. (OR = 6.3, CI = 0.7-323.2 and OR = 8.7, CI = 0.3-798.2, respectively).

Traceback Investigation

FDA conducted a traceback investigation on both lettuce and cheddar cheese. Initial supply information indicated that there were 2 different distributors and 2 different sources of cheddar cheese to the implicated restaurants. One distributor in Burlington, New Jersey covered the New Jersey/New York City area while the other in Albany, New York covered the upstate New York locations. Lettuce had a single distributor that covered all of the implicated restaurants. Further investigation of implicated lettuce lots from different restaurants traced the source of all lots to fields in California's Central Valley.

Chain A permanently removed green onions from all restaurants nationwide on December 6 and subsequently changed the produce supplier for the Northeast region.

Laboratory Investigations

The outbreak strain of *E. coli* O157:H7 was based on analysis of the PFGE pattern. The *Xba*I and *Bln*I enzyme patterns were CDC PulseNet pattern numbers EXHX01.1486 and EXHA26.007, respectively. As of December 1, 2006, the *Xba*I pattern accounted for only 20 (0.08%) of the 24,000 *Xba*I patterns in the PulseNet database while the *Bln*I pattern accounted for 22 (0.19%) of the 11,640 *Bln*I patterns. The combination of the two patterns had never been reported to PulseNet prior to this outbreak. Among the 77 cases reported, 69 matched the outbreak strain by both enzyme patterns. Further laboratory analysis performed by state health departments and CDC found the outbreak strain to be Shiga toxin 2-producing only.

A total of 384 food samples were collected from restaurants, manufacturers, and patients including samples of lettuce and cheddar cheese from implicated restaurants. Preliminary results by a private laboratory on green onions tested positive for *E. coli* O157; however, confirmatory testing by FDA was negative for this sample. A sample of white onions from NY collected from an open bin in one of the implicated restaurants yielded *E. coli* O157 but the PFGE pattern (EXHX01.3586/EXHA26.0978) did not match the outbreak strain. No other food items tested yielded *E. coli* O157. No green onion samples yielded *E. coli* O157.

DISCUSSION

A large outbreak of *E. coli* O157 infections occurred with 77 (69 confirmed and 8 probable) cases from 5 states. Fifty-five patients (72%) were hospitalized and 7 (9%) developed HUS. The outbreak was identified by local health departments based on an increase of *E. coli* O157 infections in their communities. Recognition of a multistate outbreak was dependent on communication among state health departments and CDC as well as use of the PulseNet database to link matching PFGE patterns. Efforts to enhance communication among health departments and to more promptly report PFGE patterns to PulseNet may expedite outbreak detection in the future.

Implicated restaurants were closed by local health authorities and Chain A, although it remains unclear to what extent this action had impact on preventing additional cases. The epidemic curve reflects a point source outbreak with the multistate investigation beginning toward the end of the outbreak. Due to the high volume of business in the restaurants and short shelf life of the perishable foods, it is likely that the contaminated food or foods were consumed or discarded prior to identification of the outbreak. Extensive cleaning and disinfection in implicated restaurants may have still played a role in preventing further infections through cross-contamination, though impact of this was not assessed.

A large proportion of patients (72%) were hospitalized during the outbreak including 7 (9%) patients that developed HUS. This large proportion may be related to the virulence factors of the organism. The outbreak strain was found to be Shiga toxin 2 producing only. Previous studies have demonstrated an association between Shiga toxin 2 and severity of disease.¹

Lettuce was the most likely primary vehicle based on several factors. Both lettuce and cheddar cheese were the two ingredients with the highest exposure among cases and the largest magnitude odds ratios. These two ingredients are most often served together in a variety of menu items. Collinearity among these ingredients caused difficulty in discerning between these two ingredients with multivariate analysis. As a result, the epidemiologic investigation went beyond the statistical modeling to incorporate other relevant information. Cheddar cheese was made from pasteurized milk and had two different distributors for different implicated restaurants. In fact if this information was incorporated into the analysis and cheese exposure was analyzed by the source of cheese, the cases exposure to cheddar cheese from one source was only 78%. Lettuce was a raw ingredient and its regional distribution was consistent with the outbreak area. Thus, all implicated restaurants were supplied lettuce by the same distributor. Combining this information with the case-control study results, lettuce was identified as the primary source of the outbreak.

However, cheese may have still played a role as a secondary source. In Chain A, bins of lettuce are often directly next to bins of cheddar cheese. (Figure 3) In the assembly of menu items, the same gloved hand could have transferred contaminated lettuce from one bin and then took cheddar cheese from the other bin. In the process, cross-contamination could have occurred. Thus, the difficulty in discerning between lettuce and cheese in the case-control study was likely due to their being highly correlated with each other-- from the ingredients being served together and also from possible cross-contamination between the two ingredients.

The outbreak investigation may have been affected by Chain A's announced near the onset of the investigation that it had identified green onions as the source. This announcement was based on preliminary dipstick testing of a private laboratory hired by the parent company. Specific information regarding this testing was not shared with CDC. Confirmatory testing by the FDA of the same green onion samples ultimately did not yield *E. coli* O157. We were concerned that cases would over report consumption of green onions (due to the announcement), compared to controls (since controls may be less likely to have heard the announcement) and therefore create a recall bias. However, most patrons of Chain A are unfamiliar with the exact ingredients of each

menu item and in particular with which items contain green onions. By focusing questionnaires on menu items, this recall bias was likely minimized.

The food exposure study may have been affected by other types of recall bias. Patients may have been more motivated to remember what they ate. Cases were slightly more likely to report more than one menu item with an average of 2.2 menu items reported compared to an average of 1.9 menu items reported by controls. Several menu items were very similar but had minor variations in ingredients. Interviewers commented that several patients and controls had difficulty recalling the specifics of the menu items ordered and thus affecting the exposure of ingredients entered in the database. Also, some patients and their dining companion controls recalled ordering a variety of menu items which were shared amongst each other but no specific recollection of which specific item was eaten by whom. Finally, the use of several methods to recruit controls is another limitation of the study. However, well dining companions accounted for the majority of controls and the results were similar when analysis was restricted to well dining companions only.

In this outbreak, epidemiologic evidence indicates lettuce as most likely source of the outbreak. Lettuce had the largest exposure, was shown to be associated with illness in a variety of models, had regional distribution consistent with the outbreak, had traceback of implicated lots converge to the same fields in California's Central Valley, was an uncooked ingredient, and has historically been found to be a common source of *E. coli* O157 in recent outbreaks. Further investigations should be directed toward revealing how lettuce became contaminated in order to devise interventions to prevent such outbreaks in the future.

RECOMMENDATIONS

1. Chain A
 - a. Review food supplier safety plans.
 - b. Continued education of all food handlers regarding safe handling of food and methods to prevent cross-contamination of food items.
 - c. Continue active communication with health departments, CDC, and FDA regarding ways to facilitate traceback investigation.
2. Local Health Departments
 - a. Continue active communication with state health departments regarding local outbreaks which may have larger scale potential.
 - b. Encourage healthcare providers regarding need for testing for *E. coli* O157, among other enteric pathogens, in patients with bloody diarrhea.
 - c. Continued epidemiological data gathering and analysis.
3. State Health Departments
 - a. Continued active lab-based surveillance of *E. coli* O157 infection for 4 weeks to ensure no new cases identified as part of the outbreak.
 - b. Continued epidemiological and laboratory support.
4. FDA
 - a. Continue investigations and research into mechanisms of contamination of green leafy vegetables.

- b. Continue communication with CDC and health departments to help facilitate outbreak investigations and traceback investigations.

Figure 1: Total number of cases (confirmed) by state in multistate outbreak of *Escherichia coli* O157:H7 infections, November-December 2006

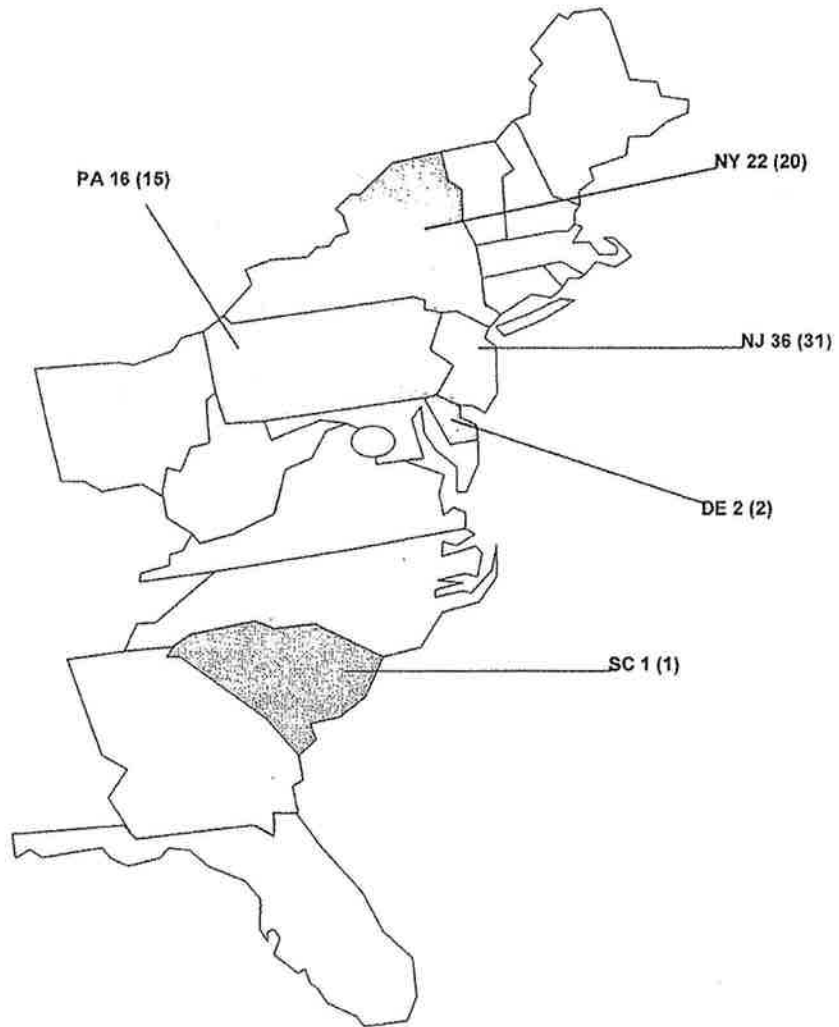


Figure 2. Location of Chain A restaurants where confirmed cases ate in multistate outbreak of *Escherichia coli* O157:H7 infections, November-December 2006.

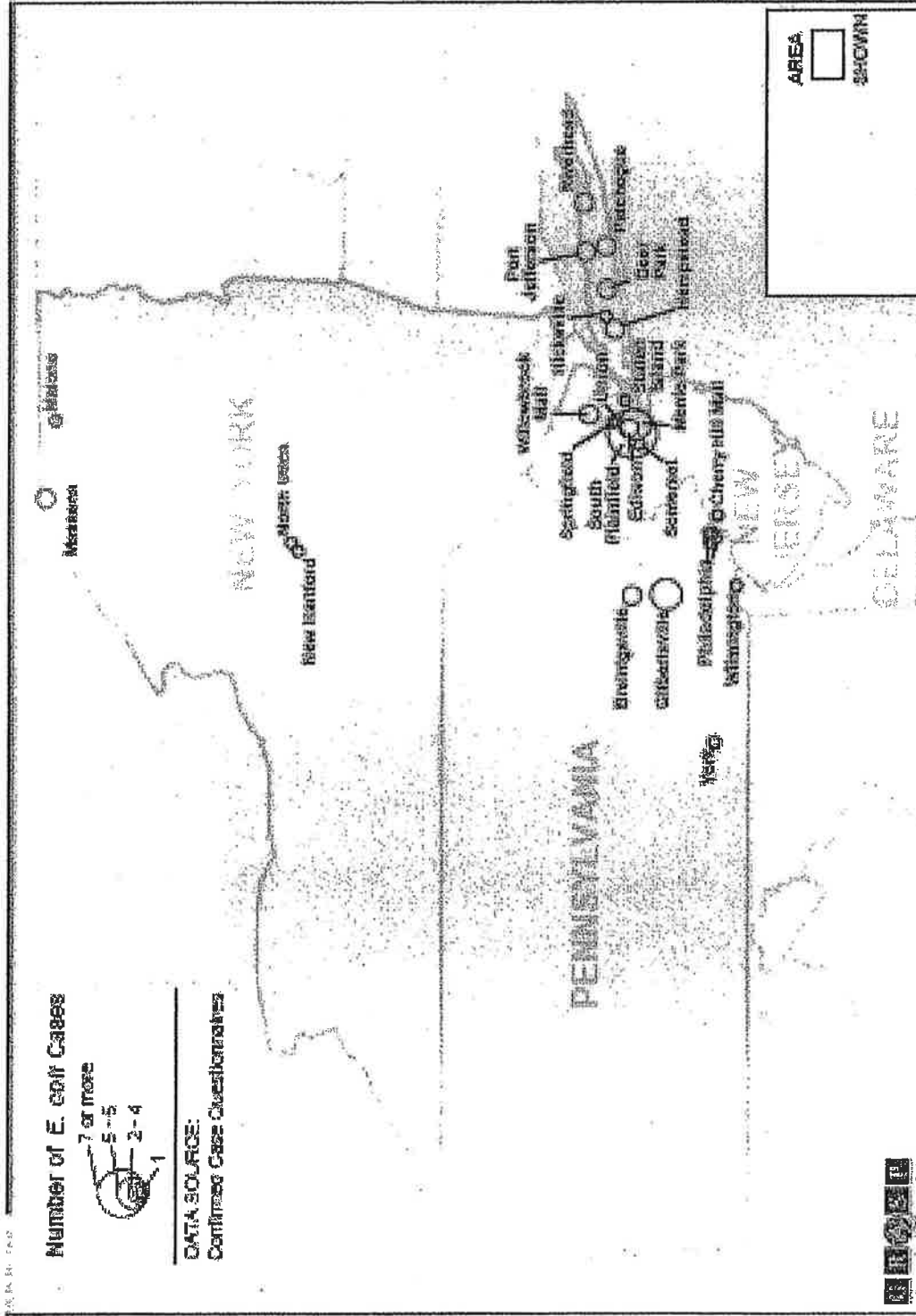


Figure 3. Number of cases by illness onset date in multistate outbreak of *Escherichia coli* O157:H7 infections, November-December 2006 (N=73).

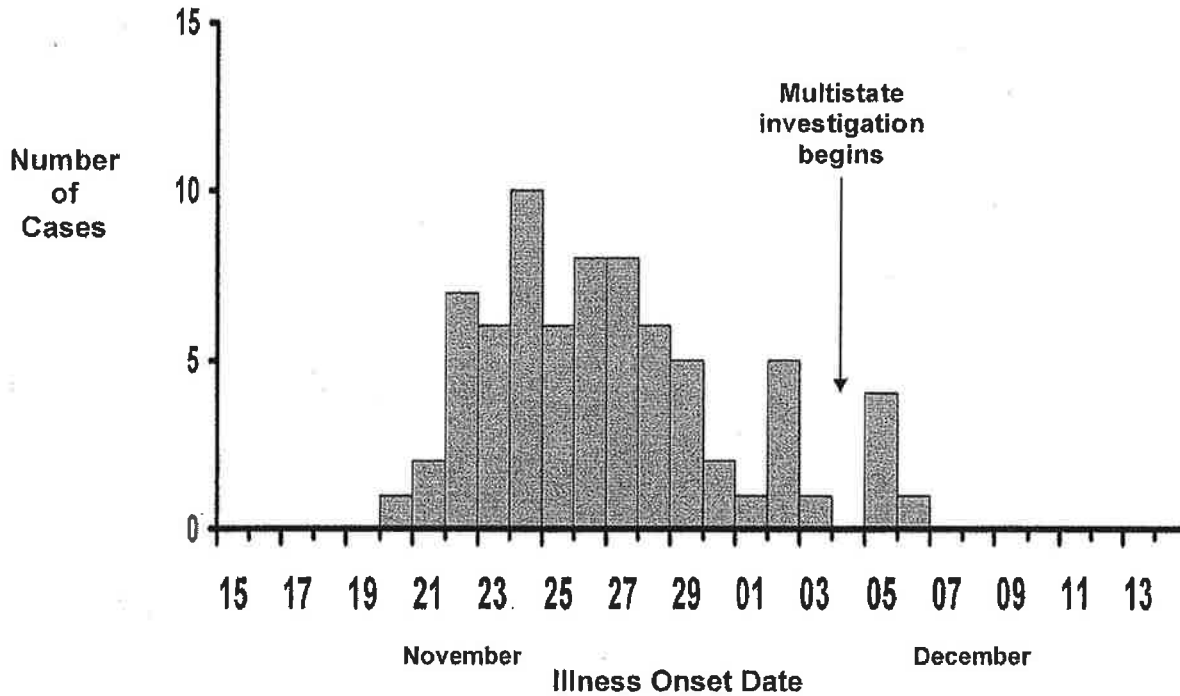


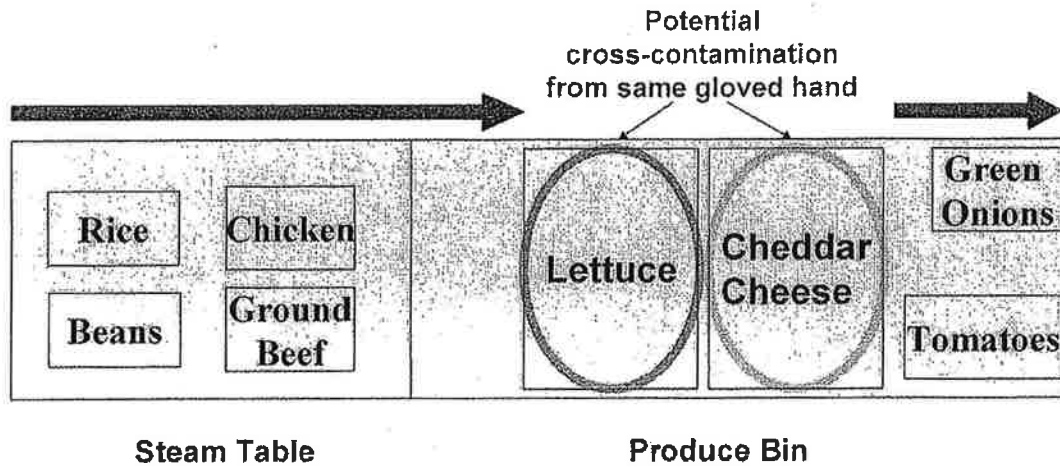
Table 1. Restaurant exposure among *E. coli* O157 cases in New Jersey and Pennsylvania with illness onset November 15-December 6, 2006.

Restaurant	Cases (n=36)	Controls (n=63)	Matched Odds Ratio (mOR)	95% Confidence Interval
Chain A	83%	3%	73.3	13.0 - Infinity
Chain B	6%	9%	0.5	0.0 - 2.9
Chain C	3%	11%	0.3	0.0 - 2.2
Chain D	0%	2%	-	0.0 - 78.0
Chain E	0%	2%	-	0.0 - 78.0
Chain X	17%	23%	0.7	0.2 - 2.5

Table 2. Ingredient exposure at Chain A among cases and controls when controlling for age (<18 yrs) in multistate outbreak of *Escherichia coli* O157:H7 infections , November-December 2006

EXPOSURE	CASES EXPOSURE (n=51)	CONTROLS EXPOSURE (n=84)	EXACT ODDS RATIO	95% CONFIDENCE INTERVAL
Lettuce	48 (94%)	61 (73%)	5.5	1.3-35.9
Cheddar Cheese	42 (82%)	43 (51%)	3.7	1.5-10.6
Ground Beef	39 (76%)	56 (67%)	2.0	0.7-6.4
Tortilla	33 (65%)	64 (76%)	0.5	0.1-1.3
Tomato	27 (53%)	40 (48%)	1.7	0.6-5.2
Sour Cream	25 (49%)	47 (56%)	0.8	0.3-2.2
Taco Shell	21 (41%)	14 (17%)	3.9	1.4-12.9
Cheese Sauce	18 (35%)	35 (42%)	0.8	0.3-2.2
X Cheese Blend	15 (29%)	38 (45%)	0.4	0.2-1.1
Chicken	14 (27%)	29 (35%)	0.5	0.2-1.4
Beans	14 (27%)	33 (39%)	0.6	0.2-1.7
Flat Bread	13 (25%)	18 (21%)	1.3	0.4-4.7
Green Onion	11 (22%)	18 (21%)	1.2	0.3-4.2
Potatoes	8 (16%)	11 (13%)	2.2	0.4-12.1
Tostada Shell	7 (14%)	13 (15%)	0.7	0.1-2.7
Chips	7 (14%)	16 (19%)	0.7	0.2-2.4
Salsa	5 (10%)	4 (3%)	5.8	0.4-332.7
Steak	4 (8%)	6 (7%)	1.9	0.3-10.7
Rice	2 (4%)	17 (20%)	0.1	0.0-0.9
Fiesta Salsa	2 (4%)	22 (26%)	0.1	0.0-0.6
Citrus Salsa	1 (2%)	0	2.0	0.1->999.9
Lime Red Strip	1 (2%)	0	2.0	0.1->999.9
Guacomole	0	1 (1%)	-	-
White Onion	0	0	-	-

Figure 3. Production line in kitchen of Chain A restaurants in multistate outbreak of *Escherichia coli* O157:H7 infections.



¹ Boerlin, P., S. A. McEwen, et al. (1999). "Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans." *J Clin Microbiol* 37(3): 497-503.