

Where's the Beef?

The Role of Cross-contamination in 4 Chain Restaurant–Associated Outbreaks of *Escherichia coli* O157:H7 in the Pacific Northwest

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Background: From March through August 1993, outbreaks of *Escherichia coli* O157:H7 occurred at 4 separate Oregon and Washington steak and salad bar restaurants affiliated with a single national chain.

Objective: To determine the cause of outbreaks of *E coli* O157:H7 at 4 chain restaurants.

Methods: Independent case-control studies were performed for each outbreak. Available *E coli* O157:H7 isolates were subtyped by pulse-field gel electrophoresis and by phage typing.

Results: Infection was not associated with beef consumption at any of the restaurants. Implicated foods varied by restaurant but all were items served at the salad bar. Among the salad bar items, no single item

was implicated in all outbreaks, and no single item seemed to explain most of the cases at any individual restaurant. Molecular subtyping of bacterial isolates indicated that the first 2 outbreaks, which occurred concurrently, were caused by the same strain, the third outbreak was caused by a unique strain, and the fourth was multiclonal.

Conclusions: Independent events of cross-contamination from beef within the restaurant kitchens, where meats and multiple salad bar items were prepared, were the likely cause of these outbreaks. Meat can be a source of *E coli* O157:H7 infection even if it is later cooked properly, underscoring the need for meticulous food handling at all stages of preparation.

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MULTIPLE foodborne outbreaks of *Escherichia coli* O157:H7 infections have been reported since the organism was first recognized as a cause of epidemic bloody diarrhea in 1982.¹ Many of these outbreaks have been attributed to consumption of undercooked beef products, including ground beef¹⁻⁴ and roast beef.⁵ Other foods, including unpasteurized apple juice,^{6,7} lettuce,⁸⁻¹² and alfalfa sprouts,¹³ have also been implicated. These produce items were apparently contaminated with *E coli* O157:H7 from environmental sources before distribution. In contrast, cross-contamination from meat to other foods during food preparation in household or commercial kitchens has not been well documented as a source of sporadic or epidemic *E coli* O157:H7 infections.

From March through August 1993, outbreaks of *E coli* O157:H7 infection occurred at 4 separate steak and salad bar restaurants in Washington and Oregon that were affiliated with a single national chain

(chain Z). In addition to serving meat, poultry, and seafood entrees, these restaurants featured large self-service salad and food bars with more than 100 items, including fresh fruits and vegetables, cold salads, pasta and sauces, and taco fixings. Some foods were purchased ready to serve, whereas others were prepared on the restaurant premises.

Surprisingly, consumption of beef or other meats was not associated with disease in any of these outbreaks. Rather, our investigations suggested that cross-contamination of various salad bar items—most likely from raw beef—occurred independently at each of the 4 restaurants.

RESULTS

The first 2 outbreaks in this series occurred concurrently in Grants Pass and North Bend, Ore, in March 1993; the third took place in Corvallis, Ore, in early August 1993; and the fourth occurred in Seattle the subsequent week in August 1993.

MATERIALS AND METHODS

EPIDEMIOLOGICAL INVESTIGATIONS

Each of the 4 outbreaks—3 in Oregon and 1 in Washington—was investigated independently by state and local public health agencies. In each investigation, a confirmed case was defined as diarrhea with a stool culture positive for *E coli* O157:H7 and a probable case as bloody diarrhea without culture confirmation. Both case definitions required onset of illness within 10 days of eating at an implicated chain Z restaurant during the appropriate outbreak period. *Escherichia coli* O157:H7 infections are reportable in Oregon and Washington; therefore, culture-positive cases were reported to the health department by health care providers or clinical microbiologic laboratories. Additional case finding was performed by active surveillance of area hospitals and clinical laboratories, notification of health care providers, and public notification through the media.

We conducted independent case-control studies for each restaurant cluster. Controls were (1) persons who had dined at the restaurant with a case but who did not become ill or (2) persons identified through credit card receipts or self-report who ate at the restaurant on the same days as cases but who did not become ill. Cases and controls were interviewed within several weeks of the outbreak using standardized questionnaires that asked about consumption of all entrees, salad and food bar items, desserts, and drinks available at the restaurant during the outbreak period.

Differences in proportions were assessed using the χ^2 statistic or the Fisher exact test when an expected cell size was less than 5.

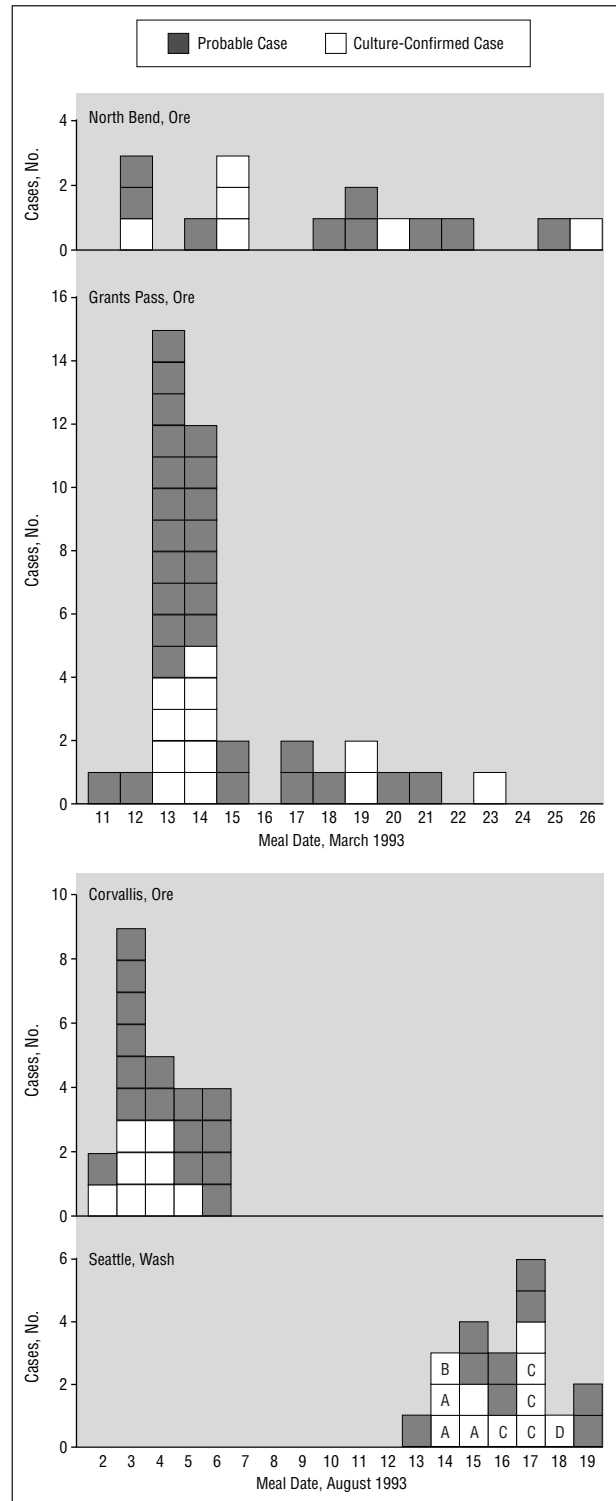
ENVIRONMENTAL INVESTIGATIONS

Local, state, and federal environmental health sanitarians inspected outbreak-associated restaurants. At the Seattle, Wash, restaurant, food samples and environmental surfaces were cultured for *E coli* O157. Product tracebacks and related inquiries were conducted when indicated.

LABORATORY METHODS

Stool culture isolates were obtained from clinical laboratories and confirmed as *E coli* O157:H7 by standard methods.¹⁴ *Escherichia coli* O157:H7 isolates were subtyped by pulse-field gel electrophoresis (PFGE) after digestion with *Xba*I as previously described¹⁵ and by phage typing.¹⁶ Most isolates were also tested by bacteriophage lambda-generated restriction fragment length polymorphism (λ -RFLP)¹⁷ and by Shiga-like toxin RFLP.^{17,18}

At each restaurant, exposures occurred during multiple days (**Figure**). We identified 39 culture-confirmed and 54 probable cases in these outbreaks. Cases ranged in age from 3 to 87 years. Fifteen cases were hospitalized, none developed the hemolytic uremic syndrome or thrombotic thrombocytopenic purpura, and none died.



Reported meal dates for *Escherichia coli* O157:H7 cases associated with outbreaks at 4 chain Z restaurants in 1993. Letters indicate the pulse-field gel electrophoresis subtypes of case isolates from the Seattle, Wash, outbreak.

CASE-CONTROL STUDIES

Consumption of meat or poultry was not associated with disease in any of the outbreaks. Among salad bar items, no single item was implicated in all of the outbreaks, and no single item seemed to explain most of the cases at any

Food Items Associated With Confirmed Cases of *Escherichia coli* O157:H7 in 4 Steak and Salad Bar Chain Restaurant–Associated Outbreaks in the Pacific Northwest, 1993

Restaurant Location	Cases, No.*	Controls, No.	Food Item	Ate Food Item, No. (%)		Odds Ratio (95% Confidence Interval)	P
				Cases	Controls		
Grants Pass, Ore	39	42	Mayonnaise-containing foods†	32 (82)	25 (59)	3.1 (1.0-10.0)	.03
			Blue cheese salad dressing	10 (26)	2 (5)	6.9 (1.3-67.9)	.01
			Carrots	11 (28)	3 (7)	5.1 (1.2-30.5)	.01
			Seafood salad	5 (13)	0 (0)	Undefined	.02
			Taco chips	5 (13)	0 (0)	Undefined	.02
North Bend, Ore	13	18	Mayonnaise-containing foods†	13 (100)	12 (67)	Undefined	.03
			Cantaloupe	7 (54)	3 (17)	5.5 (0.9-44.5)	.05
Corvallis, Ore	24	44	Tomatoes	13 (54)	12 (27)	3.15 (0.98-10.4)	.05
			Cantaloupe	13 (54)	10 (23)	4.0 (1.2-13.7)	.02
			Tostadas	12 (50)	9 (21)	3.9 (1.15-13.5)	.02
Seattle, Wash	20	26	Lettuce	17 (85)	15 (58)	4.2 (1.0-21.0)	.04
			Prepackaged cheese food product	12 (60)	5 (19)	3.9 (1.1-14.6)	.02

*Defined by isolation of *E coli* O157:H7 from stool samples or report of bloody diarrhea without laboratory confirmation.

†Foods prepared on-site from mayonnaise distributed to the restaurant in bulk containers.

individual restaurant (**Table**). Food items associated with illness in the various investigations included cantaloupe, mayonnaise-containing foods, lettuce, and bulk prepackaged shredded cheese food product.

The Grants Pass and North Bend outbreaks overlapped in time (Figure). These case-control studies implicated several items prepared on the premises with bulk mayonnaise, including imitation crab and macaroni salads. Mayonnaise-containing foods that were prepared off-site (eg, potato salad) were not associated with illness.

SUBTYPING OF ISOLATES

The 15 isolates from the Grants Pass and North Bend clusters that were available for testing were not typeable by PFGE (all produced a smear), but all were indistinguishable by phage typing (phage type 31). Nine of these isolates were also tested by λ -RFLP¹⁷ and by Shiga-like toxin RFLP,^{17,18} and all had the same RFLP pattern. All of the Corvallis outbreak isolates tested (n=13) were indistinguishable from each other by PFGE and produced a distinct banding pattern that allowed comparison with other subtyped isolates.

In contrast to the clonal nature of the isolates from the Oregon outbreaks, the 9 isolates tested from the Seattle outbreak were grouped into distinct subtypes by the methods used. By PFGE, 4 distinct band patterns, arbitrarily designated A (3 isolates), B (1 isolate), C (4 isolates), and D (1 isolate) were identified (Figure). These 4 patterns differed from each other by at least 3 bands. Subtyping by RFLP methods produced equivalent results.

We also subtyped by PFGE selected Washington (n=101) and Oregon (n=44) isolates that were not known to be epidemiologically linked to the 4 chain Z outbreaks, including several from persons with a history of eating at other chain Z restaurants before onset of their illness. Only 1 of these isolates matched any of the outbreak patterns. That isolate, which was indistinguishable from the Grants Pass/North Bend isolates by PFGE and by λ -RFLP and Shiga-like toxin RFLP, came from a

human immunodeficiency virus–infected man who reported eating a steak and a potato (but no salad bar items) at a Portland, Ore, chain Z restaurant 3 days before onset of his illness on March 22, 1993.

Pulse-field gel electrophoresis subtyping of isolates of *E coli* O157:H7 from 2 persons who reported eating at different, non-outbreak-associated, chain Z restaurants in western Washington before their illness, and who had onset of illness in August or September 1993, revealed that each isolate was unique and both were distinct from all of the chain Z outbreak-associated isolates tested.

ENVIRONMENTAL INVESTIGATIONS

For at least 2 restaurants, inspections revealed several violations of applicable food codes and kitchen designs that were less than ideal in that raw meat was being processed and stored in close proximity to raw vegetables and other food products. No direct evidence of improper food handling that could have caused these illnesses was apparent, however. No employee reported any history of gastrointestinal tract illness preceding the respective outbreaks. *Escherichia coli* O157:H7 was not isolated from any of the more than 40 food items and surfaces sampled at the Seattle restaurant.

A single meat distributor supplied all 4 of the outbreak-associated restaurants, as well as the Portland restaurant patronized by the man with the matching isolate. Although some cuts of meat were supplied to the restaurants in individual-sized portions, most meat items were trimmed, cut, and tenderized on-site from beef “tri-tips” that typically arrived in approximately 5-kg vacuum-sealed packages. The tri-tips delivered to all 4 outbreak-associated restaurants came from a single meatpacking plant that was 1 of at least 9 suppliers to chain Z restaurants nationwide at the time. A more extensive traceback investigation of the meat sources was not performed. At the restaurants, the meat was tenderized by maceration in a jacquard machine, which consists of multiple needle-like spikes, and then marinated before cooking. Mul-

tiple steaks were marinated in a common pan, which was refrigerated with other perishable packaged and fresh food products.

With the exception of meat and some dry goods, there was no common distributor of produce or other fresh foods to all 4 restaurants. Bulk mayonnaise from the same manufacturer was delivered to the North Bend and Grants Pass restaurants in 13.5-kg boxes, but there was no evidence of inadequate manufacturing safeguards or contamination at the mayonnaise factory. This mayonnaise had been held at ambient temperatures for more than 2 months before distribution. Delivery truck drivers and restaurant employees denied seeing any evidence of leaking, wet, or otherwise damaged mayonnaise boxes. No illness was identified that could be linked to other institutions that received mayonnaise, fruit, or other implicated items from the same suppliers and production or distribution lots as the 4 outbreak-associated restaurants.

COMMENT

A remarkable series of *E coli* O157:H7 outbreaks occurred at 4 chain Z restaurants in Oregon and Washington between March and August 1993. Although beef featured prominently on the menu of these steak and salad bar restaurants, consumption of meat was not associated with illness in any of the outbreaks. Implicated food items varied by restaurant, but all were foods or condiments served at the salad bars.

Why did these outbreaks occur? Our investigations, although not conclusive, strongly suggest that similar but independent events of cross-contamination from uncooked beef to other foods within each of the 4 restaurants led to the outbreaks. This is supported by the fact that all 4 restaurants obtained their raw beef, which is a well-documented source of *E coli* O157:H7,^{2-5,19,20} from the same meatpacker, which was one of several that supplied chain Z restaurants nationwide. The first 2 outbreaks, which occurred simultaneously and yielded indistinguishable bacterial isolates, occurred at restaurants that did not share a common source of produce but did share meat delivered from the same truck on the same delivery run. Moreover, the implicated food items varied by restaurant, suggesting either that the restaurants coincidentally received different foods contaminated with *E coli* O157:H7 during a fairly narrow time interval or that the foods were contaminated independently within each restaurant from contaminated meat that was more widely distributed. No cases linked to consumption of implicated nonbeef food items (ie, mayonnaise, cantaloupe, and lettuce) at other non-chain Z institutions were identified. Last, the only matching *E coli* O157 isolate identified among the “background” isolates tested came from a patient with a history of eating beef at a fifth chain Z restaurant supplied by the same meat distributor.

No single food was implicated as a source of any of the 4 outbreaks, perhaps because of the inherently limited capacity of retrospective investigations to identify a single source when a large number of items are served, many with shared ingredients. In addition, multiple items might have become contaminated in at least some of the outbreaks, either by kitchen staff during preparation or

possibly by restaurant patrons after the items had been placed in the salad bar. This scenario is perhaps most plausible for the Seattle outbreak, the multiclonal nature of which suggests that more than 1 cross-contamination episode might have occurred. This hypothesis is supported by the clustering of the dates of the restaurant visits for persons infected with isolates of the same subtype. Alternatively, however, a single food item could have been contaminated by multiple isolates, possibly from marinade that contained juices from multiple cuts of meats. If a single item was the source for any of the outbreaks, the range of dates of exposure for the associated cases indicates that it must have been served over a fairly prolonged interval.

We considered the possibility of intentional contamination—a rarely reported cause of foodborne outbreaks.^{21,22} Although impossible to rule out, we believe that the heterogeneity of outbreak-associated isolates, the identification of a matching case at the Portland restaurant, and the presence of a plausible alternative scenario combine to make sabotage an unlikely explanation. A private investigation sponsored by chain Z came to a similar conclusion.

Several items implicated in these investigations had not previously been identified as vehicles for *E coli* O157:H7. Notwithstanding its popular reputation as the classic vehicle for foodborne illness, the bactericidal properties of mayonnaise—due in large part to its low pH—have long been appreciated by food scientists.²³ Results of earlier experiments using apple cider suggested that *E coli* O157:H7 may be unusually acid tolerant,⁶ however, and experiments conducted subsequent to the chain Z outbreaks confirmed that *E coli* O157:H7 inoculated into mayonnaise can survive for months at refrigerated temperatures.²⁴⁻²⁶ At higher temperatures, such as those obtained at the mayonnaise factory, however, survival is limited to at most a few days, indicating that contamination must have occurred after the mayonnaise left the factory. Fresh cantaloupe and tomatoes have been implicated in outbreaks of salmonellosis,²⁷ and experiments conducted after the Corvallis outbreak confirm that they can readily support growth of *E coli* O157:H7.²⁸

Since the Seattle outbreak, lettuce has been implicated as a source of several *E coli* O157:H7 outbreaks,⁸⁻¹² raising the possibility that it might have been a direct source for this particular cluster. Although a large proportion of the Seattle cases consumed lettuce, this was highly correlated with consumption of other salad bar items, making it difficult to determine whether lettuce was independently associated with illness. Furthermore, the rapid turnover of lettuce at the salad bar and its perishability make it an improbable source for an outbreak that lasted at least 7 days.

An obvious question is why chain Z restaurants in particular were repeatedly affected. Although cross-contamination events are difficult to confirm retrospectively, we speculate that the practice of trimming, macerating, and marinating the beef tri-tips in the same kitchens used for preparation of fruits, vegetables, and other salad bar items might have enhanced the potential for spatter or spillage of meat juices. According to anecdotal information from company officials and public

health restaurant inspectors, chain Z may have been exceptionally susceptible to cross-contamination because on-site meat cutting and large, diverse salad bar operations were combined. If, by chance, these chain Z restaurants received lots of beef that were heavily contaminated with *E coli* O157, these circumstances would have amplified the probability of the occurrence of an outbreak. Following these outbreaks and an outside review of their food-handling practices, chain Z instituted a comprehensive Hazard Analysis and Critical Control Point program. Chain Z-owned restaurants changed from using on-site meat cutting to using precut meat, as did many franchised restaurants. To our knowledge, no subsequent outbreaks or sporadic cases of *E coli* O157:H7 infections have been linked to chain Z restaurants.

Molecular subtyping has been increasingly used to augment not only outbreak investigations^{3,7} but also routine surveillance for foodborne illnesses.^{29,30} Subtyping results may suggest natural groupings of cases, facilitating efforts to identify potential common-source exposures. Alternatively, when cases are clustered temporally and geographically, suggesting the occurrence of an outbreak, but multiple diverse strains are found by subtyping, further investigation to identify a common source may not be indicated.

At the time of these outbreaks, routine subtyping of *E coli* O157:H7 isolates was not being done in Oregon or Washington. Consequently, none of the chain Z outbreaks were first identified by subtyping. These common-source restaurant exposures were readily identified from routine surveillance interviews conducted by public health officials. In the first 3 outbreaks, subtyping subsequently indicated that the cluster-related isolates seemed to be homogeneous, which is typical of most common-source *E coli* O157 outbreaks.^{3,4,13,15} The Seattle outbreak, however, is a striking exception to this rule and serves as a cautionary reminder that subtyping is an adjunct to epidemiological investigation, not a substitute for it. Categorization of the Seattle isolates by subtyping alone would not have indicated that they were related to a common source. At least one other multiclonal *E coli* O157:H7 outbreak has been reported,³¹ and it involved transmission via unpasteurized milk from a colonized herd over an approximately 18-month period.

Our investigations suggest that relatively subtle lapses in food-handling procedures might be sufficient to result in an outbreak of *E coli* O157:H7 infections, likely a consequence of the relatively low infectious dose of this organism.^{19,32} Through cross-contamination, meat can be a source of *E coli* O157:H7 infection even if it is later cooked properly. Although other measures to prevent *E coli* O157:H7 infection are being evaluated, such as vaccines³³ and modifications in cattle feed composition,³⁴ currently, avoidance of consumption of contaminated food is the only preventive measure available, underscoring the need for meticulous food handling at all stages of preparation in commercial and home settings.

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