# Investigation of an Outbreak of Gastrointestinal Illness at the Mayfair Road Sizzler Restaurant, Wauwatosa, WI; July – August, 2000

Final Report

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## ABBREVIATIONS

| | |
|---|---|
| BCD | Bureau of Communicable Diseases |
| BEH | Bureau of Environmental Health |
| CDC | Centers for Disease Control and Prevention |
| CDES | Communicable Disease Epidemiology Section |
| CFSAN | Center for Food Safety and Nutrition |
| *E. coli* | *Escherichia coli* |
| FDA | Food and Drug Administration |
| FSIS | Food Safety and Inspection Service |
| LASR | Layton Avenue Sizzler Restaurant |
| MRSR | Mayfair Road Sizzler Restaurant |
| MHD | City of Milwaukee Health Department |
| MHDL | Milwaukee Health Department Laboratories |
| PCR | Polymerase chain reaction |
| PFGE | Pulsed-field gel electrophoresis |
| RT-PCR | Reverse transcriptase polymerase chain reaction |
| USDA | United States Department of Agriculture |
| WHD | Wauwatosa Health Department |
| WDATCP | Wisconsin Department of Agriculture, Trade, and Consumer Protection |
| WDPH | Wisconsin Division of Public Health |
| WSLH | Wisconsin State Laboratory of Hygiene |

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## BACKGROUND

On August 2, 2000 during the Layton Avenue Sizzler Restaurant (LASR) *E. coli* O157:H7 outbreak investigation, two individuals who denied eating at the LASR during July had laboratory-confirmed *Escherichia coli (E. coli)* O157:H7 isolates that matched the LASR outbreak strain by pulsed-field gel electrophoresis (PFGE). Both individuals reported eating at the Mayfair Road Sizzler Restaurant (MRSR) on July 16, 2000 and experiencing onset of signs and symptoms on July 19 and 24. The immediate investigation response plan on August 2 included a decision to send the Wauwatosa health officer and two Wauwatosa Health Department (WHD) sanitarians into the MRSR at 5 pm that evening to explain the situation to the manager, to inspect the restaurant, and to collect food samples for testing.

## METHODS

### Early control measures:

During the evening of August 2, the manager of the MRSR voluntarily closed the restaurant. Food samples were collected from the buffet lines and the walk-in coolers by the WHD sanitarians and were secured overnight in a refrigerator in a locked room at the WHD. Other food products on the premises were ordered held pending completion of the investigation.

### Surveillance:

***Case finding***

Press releases On August 3, 2000 the WHD and the City of Milwaukee Health Department (MHD) issued a joint press release **(appendix A)** describing the two MRSR-associated *E. coli* O157:H7 infections and their relationship to the LASR investigation. The press release requested that persons who ate at the MRSR during July 14 to 18 call the Wisconsin Division of Public Health (WDPH), Bureau of Communicable Diseases (BCD) Communicable Disease Epidemiology Section (CDES) to be interviewed. Seven additional joint press releases were issued by the WHD and MHD between August 7 and 24, 20000.

Laboratory surveillance Additional potential cases of *E. coli* O157:H7 infection associated with the MRSR were sought by review of laboratory reports from the Milwaukee Health Department Laboratories (MHDL), Children's Hospital of Wisconsin Laboratory, Medical Sciences Laboratory, St. Francis Hospital Laboratory, St. Mary's Laboratory, Waukesha Memorial Hospital Laboratory, Aurora Clinical Laboratory, and the Wisconsin State Laboratory of Hygiene (WSLH).

PulseNet posting On July 28, the WSLH PFGE laboratory posted the outbreak pattern on the Centers for Disease Control and Prevention (CDC) PulseNet system **(appendix B)** to allow other states conducting PFGE of *E. coli* O157:H7 isolates from their state residents to identify specimens with matching PFGE patterns.

**Epidemiologic survey :**

***Case control study***

An outbreak-specific case-control interview form **(appendix C)** was developed by CDES staff which included demographic data, information about signs and symptoms of disease, consumption of 140 food items listed on the MRSR menu and questions regarding other known risk exposures for *E. coli* O157:H7 infection. Individuals who called the CDES to report signs and symptoms of illness within seven days of eating at the MRSR between July 9 and August 2 were interviewed with the case-control interview form. Community and family controls who ate at the MRSR during this same time frame but who did not experience signs or symptoms of illness were interviewed using the same case-control interview form as the symptomatic individuals. Community controls were identified from phone calls from Wisconsin residents to the CDES. Family controls were identified when symptomatic individuals were interviewed.

**Laboratory Investigation :**

***Bacteriologic and molecular analysis of human specimens***

Stool specimen kits were supplied by the WSLH and distributed by the WHD for all MRSR employees and for symptomatic potential restaurant-associated ill persons who did not have a primary care physician that could provide testing. Raw stool specimens were also collected by WDPH regional staff the weekend of August 5 and 6 from nine individuals with recent onset of signs and symptoms. These nine stool samples were first tested at the WSLH for Calicivirus and later forwarded to the Respiratory and Enterovirus Branch, National Center for Infectious Diseases at CDC for Norwalk-like virus polymerase chain reaction (PCR) testing.

Wisconsin State Laboratory of Hygiene The WSLH tested MRSR employee stools for *E. coli* O157:H7 infection. Stool specimens were screened for *E. coli* O157:H7 using MacConkey sorbitol medium (Difco Laboratories, Detroit, MI), and sorbitol negative colonies were confirmed as *E. coli* O157:H7 using O157 and H7 agglutination tests. Individual case-patient *E. coli* O157:H7 isolates were forwarded to the WSLH for PFGE analysis.

The PFGE method was conducted by standard technique (Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention. PulseNet – The national molecular subtyping network for foodborne and disease surveillance. One day standard laboratory protocol for molecular subtyping of *E. coli* O157:H7 by pulsed-field gel electrophoresis, 1998). Genomic DNA in agarose plugs was restricted with Xba1 and Bln1 as recommended by CDC's PulseNet procedure. The resulting macrorestriction fragments were separated by CHEF-PFGE using a CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, CA) at 200 V and $14^0$C for 18 to 22 hours with switching times ranging from 2.16 to 54.17 seconds. E. coli G5244 was used as the reference standard. Molecular Analyst-Finger Printing Plus software (Bio-Rad) was used for analysis of restriction bands with normalization to the PulseNet Global Standard provided by CDC.

The WSLH performed PCR testing of raw stool samples from individuals for Calicivirus using a reverse transcriptase method using "region B" primers specific for Calicivirus. Specimens from this outbreak investigation which were negative were forwarded to CDC for expanded Norwalk-like virus testing.

Centers for Disease Control and Prevention The Respiratory and Enterovirus Branch Laboratory at CDC performed PCR testing of raw stool samples from nine individuals that were tested at the WSLH for Calicivirus and were negative. These nine stool specimens were tested for the whole family of *Caliciviridae* including "Norwalk-like viruses" using a recently developed reverse transcriptase-polymerase chain reaction (RT-PCR) with "region a" and "region b" primers. Specimens initially positive were further characterized by determining the nucleotide sequence of the reverse transcriptase polymerase chain reaction (RT-PCR) product and comparing this sequence to those Norwalk-like virus strains in the CDC database. This test procedure is experimental and has not been approved or licensed by Food and Drug Administration (FDA).

***Laboratory analysis of food samples***

Various food items from the buffet line collected at the MRSR during restaurant inspections were evaluated at two or more laboratories for various pathogens. Foods were examined for the presence of *E. coli* O157:H7 bacteria at the Wisconsin Department of Agriculture, Trade and Consumer Protection (WDATCP) laboratories, and some were also tested for *E. coli* O157:H7 at the United States Department of Agriculture (USDA) laboratory. Chicken wings and sirloin tri-tip samples were evaluated at the WSLH for toxin-producing *E. coli*.

Wisconsin Department of Agriculture, Trade, and Consumer Protection Laboratories The WDATCP tested food samples collected by WHD staff from the food buffet lines at the time of restaurant inspections. The WDATCP also tested unopened packages of food items in the walk-in cooler and freezer collected during a series of restaurant inspections during the outbreak.

United States Department of Agriculture Two USDA inspectors collected samples from the Mayfair Road Sizzler on August 2 and August 3 and forwarded them to the USDA laboratory in Athens, GA for pathogen analysis.

Milwaukee Health Department Laboratory The MHDL tested intact watermelons from the MRSR using an FDA method provided by Sherry McGarry from the CFSAN outbreak investigation team for detection of *E. coli* O157:H7 on the rind and interior of melons.

**Environmental Investigation :**

The WHD participated in a series of conference calls with CDC, FDA, USDA, and CDES, on August 2, 3, 4 and 8. Questions arose regarding the grinding process (raw vs. cooked), the preparation of taco meat, rib eye food history, and sources of watermelons. The USDA conducted traceback and trace forward of meats. Potential cross-contamination was discussed. The WDATCP analyzed Sysco invoices provided by WHD and MHD to determine meat and melon

similarities and to determine if there was any movement between the MRSR and LASR.

***Restaurant inspection and sample collection***

Food samples collected the evening of August 2 were transported in portable coolers on August 3 to the WDATCP laboratories. On August 3, two federal USDA compliance officers collected samples of beef and poultry and forwarded the samples to their laboratory in Athens, GA for pathogen testing. On August 4 WHD sanitarians collected intact watermelon, honeydew and cantaloupe melons and cubed raw beef from the restaurant. The intact watermelon was delivered to the MHDL. WHD sanitarians also took pictures of the walk-in cooler, dry storage room, cooking line, dishwashing area, the small food prep room, and the large food prep area. A diagram of the restaurant floor plan was sketched. The restaurant management team and members of WHD met several times at the restaurant during the next two weeks to review food preparation processes and procedures and to look at the physical layout of the establishment. Invoices of food purchased during July were obtained to facilitate any traceback of food that would be necessary. Environmental swabs were collected from the grinder/mixer, vegetable chopper, lettuce cutter, and the slicer on August 9. The WHD worked cooperatively with the BCD, MHD, WDATCP, USDA, and FDA during the investigation.

***Employee interviews and specimen collection***

Between August 8 and August 11, 2000, MRSR employees were interviewed by WHD and CDES staff using a standardized interview form **(appendix D)**. Employees were also asked about food preparation methods and provided with a #10 stool specimen kit for pathogen analysis. Stool specimen kits were provided by the WHD to employees of the MRSR and forwarded to the WSLH for pathogen screening (see laboratory investigation). Employee interviews were evaluated and summarized by CDES staff.

**Data analysis:**

Analysis of case-control data was conducted by CDES staff using Mantel-Hanszel matched chi-square analysis for dichotomous variables (Epi-Info, version 6.0; CDC, Atlanta, GA). Ninety-five percent confidence intervals for odds ratios were calculated using the method of Cornfield; probability values (p value) were calculated by Fishers' 2-tailed exact test. Multivariate analysis was performed using Statistics® analytic software (Tallahassee, FL) and was based on Mantel-Hanszel methodology.

## RESULTS

### Surveillance:

***Case finding***

Two laboratory-confirmed cases of *E. coli O*157:H7 infection were identified as associated with eating at the MRSR during July 9 to 27, 2000 **(appendix E, figure 1)**. Both had eaten at the Mayfair Road restaurant on July 16. Onset of signs and symptoms began on July 19 for the one year old male and on July 24 for the 69 year old female **(appendix F, figure 2)**. Among the approximately 1700 calls received by the MHD in association with the LASR, only these two individuals with laboratory-confirmed for *E. coli O*157:H7 infection were identified in association with the MRSR. In addition to the two laboratory-confirmed cases of *E. coli O*157:H7 infection, phone calls from patrons of the MRSR identified 50 additional individuals with onset of signs and symptoms of gastrointestinal illness within 15 days of eating at the restaurant.

### Epidemiologic findings:

***Descriptive epidemiologic features***

Among the 52 symptomatic patrons of the MRSR, the most frequently reported signs and symptoms included diarrhea (96%), abdominal cramps (94%), fatigue (77%), and nausea (54%) **(appendix G Table 1)**. The mean incubation period for all 52 individuals was 3.8 days (median 3.0; range 0.5 to 15 days) and duration of diarrhea was 5.5 days (median 4.0 days; range 1 to 14 days). Among these 52 individuals with signs and symptoms, 19 (37%) had onset of signs and symptoms less than two days after eating at the restaurant while the other 33 (63%) experienced signs and symptoms two or more days after eating at the MRSR. A summary of the frequency of signs and symptoms for these two different incubation periods is summarized in **Table 1, appendix G)**. The reported signs and symptoms among persons in these two groups were similar except that those in the longer incubation period group experienced a higher frequency of fatigue (82% vs. 68%) and bloody diarrhea (24% vs. 11%) than the shorter incubation period group.

Restaurant exposure dates for the 19 individuals with shorter incubation times (< 2 days) included the 12 day period July 11 to July 22 while exposure dates for the 33 individuals with longer incubations times (≥ 2 days) included the 19 day period July 9 to July 27. Community and family controls included in the case-control study dined at the restaurant between July 10 and July 27 **(appendix H, Figure 3)**. Dates of onset of signs and symptoms for the 19 restaurant patrons with shorter incubation period were July 12 to July 23 and included July 13 to August 1 for the 33 restaurant patrons with longer incubation periods **(appendix I, Figure 4)**.

***Case-control study***

Case-control interviews included over 140 food items on the MRSR menu. In univariate analysis both lettuce and chicken wings were associated with illness whether the analysis included all 52

symptomatic persons, only shorter incubation individuals, or only longer incubation individuals **(appendix J, Table 2).** A multivariate analysis of all ill patrons indicated that lettuce and chicken wings were independently associated with illness. When the 19 restaurant patrons with shorter incubation periods were evaluated in multivariate analysis, illness was associated with consumption of lettuce (OR = 11.75; 95% CI 2.1, 65.83; p=0.0051) but not with chicken wings. When the 33 individuals with the longer incubation periods were evaluated in multivariate analysis, illness was associated with consumption of chicken wings (OR = 8.11; 95% CI 1.89, 34.75; p = 0.0048) but not with lettuce. The full attack rate table for the case-control study at the MRSR is included as **appendix K**. The epidemiologic study did not link illness with eating at other restaurants or with other known risk factors associated with previously reported outbreaks of *E. coli* O157:H7 including consuming unpasteurized milk, contact with cattle or cattle manure, recreational water exposure, diaper-changing, or contact with an individual with known *E. coli* O157:H7 infection.

**Laboratory investigation**:

***Bacteriologic and molecular testing of human specimens***

Case-patients The two MRSR *E. coli O157:H7* MRSR case-patient isolates were indistinguishable by PFGE (using both Xba1 and Bln1 enzymes) from the following: each other, 60 *E. coli O157:H7* isolates from cases associated with the LASR, the raw chunky taco meat *E. coli O157:H7* isolate from the LASR, and the *E. coli* O157:H7 isolates from the intact cryopac sirloin tri-tips from the same lot (XL EST 86R) that was supplied to the MRSR and LASR during the outbreak period (**appendix L**). In addition, 26 other *E. coli* O157:H7 specimens received at the MHDL and WSLH during the outbreak period from individuals who had not eaten at the MRSR or the LASR had PFGE patterns which were different from each other and from the Sizzler outbreak pattern. A dendrogram detailing PFGE results of 51 of the outbreak-related *E. coli* O157:H7 isolates and 6 of the non-outbreak isolates is included as **appendix M.**

Among the 50 symptomatic patrons of the MRSR who did not have a laboratory-confirmed *E. coli* O157:H7 infection, stool specimens from nine individuals with the most recent onset of signs and symptoms were tested at the WSLH: all nine were negative for Calicivirus (personal communication, Carol Kirk, WSLH). These nine stool specimens were forwarded to the Respiratory and Enterovirus Branch Laboratory at CDC where the results of Norwalk-like virus RT-PCR testing are pending.

Restaurant employees Among the 41 employees of the MRSR, 24 (59%) submitted stool samples for culture between August 8 and August 19 **(appendix N)**. All 24 stool samples were negative for *E. coli* O157:H7. None of the stools was tested for Calicivirus.

***Testing of environmental and food samples***

Milwaukee Health Department Laboratory Environmental swabs collected from the grinder/mixer, vegetable chopper, lettuce cutter, and the slicer at the MRSR on August 9 were tested at the Milwaukee Health Department Laboratory for pathogens: all were negative (personal

communication, Dr. Singh, Milwaukee Health Department Laboratory).

Wisconsin Department Of Agriculture, Trade, and Consumer Protection Laboratories
During the MRSR outbreak investigation, a total of 31 food samples were taken from the restaurant and tested by WDATCP. Among the food samples tested, one was positive and five were weakly positive using the *E. coli* Reveal® test but all were negative on confirmatory tests **(appendix O and P)**.

Wisconsin State Laboratory of Hygiene Among the 31 food samples taken from the MRSR during the investigation, the WSLH tested eight chicken wings and sirloin tri-tips food items for shiga-toxin producing *E. coli* using a multiplex PCR assay. MacConkey plates were swept and individual colonies were evaluated. None of the food items was positive for shiga-toxin producing *E. coli* but most of the samples produced malate dehydrogenase which indicates the presence of some strain of *E. coli* or related enterobacteria but not in conjunction with shiga-like toxin or adhesion genes (personal communication, Dr. Peter Evans, Emerging Pathogens Fellow, WSLH).

United States Department of Agriculture Traceback information from MRSR and LASR invoices for the month of July indicated that both restaurants had obtained raw meat products from Excel Corporation, Wichita, Kansas, during the outbreak period. The USDA secured and tested a total of 22 meat products associated with lot numbers shipped to the restaurants during the outbreak period: 8 were obtained from the Food Safety and Inspection Service (FSIS) plant, 10 were collected at Wisconsin state plants, and 4 were Sizzler restaurant samples. None of the 22 specimens tested by the USDA laboratory was positive for *E. coli* O157:H7 **(appendix Q)**. An intact cryovac sirloin tri-tips roast (with the same lot # XL EST 86R as invoices indicated were at the MRSR and LASR at the time of the outbreak) was obtained by USDA staff from the LASR walk-in cooler on August 1 and forwarded to Summerfry Labs (SF) for pathogen isolation. SF labs identified two *E. coli* O157:H7 isolates from this intact sirloin tri-tips sample **(appendix Q)**. These two *E. coli* O157:H7 isolates from the intact sirloin tri-tips (BC 5674 & BC 5677) were forwarded to the WSLH where the PFGE pattern was found to be indistinguishable from the *E. coli* O157:H7 isolate from the raw chunky taco meat from the LASR (BC 5033), the LASR outbreak-related patient isolate pattern (BC 5028 & BC 5029), and the MRSR patient isolate pattern (BC 5131) **(appendix L)**.

Food and Drug Administration After the association of illness with watermelons was identified in the LASR case-control study, intact watermelons in the MRSR and LASR walk-in coolers were tested by the MHDL using an FDA method provided by the CFSAN outbreak investigation team for detection of *E. coli* O157:H7 on the rind and interior of melons. Samples from one uncut watermelon obtained on July 14, 2000 were shared with FDA and Summer Fry laboratories. The screening test for all watermelons was negative at the MHDL **(appendix R)** and at FDA (personal communication, Clifford Purdy, FDA liaison to CDC).

**Environmental investigation**:

***Employee interviews***

Between August 8 and August 16, 2000, 36 (88%) of the 41 employees of the MRSR were interviewed. This included 12 servers, 9 utility workers (dishwashers), 6 salad bar workers, 5 cooks, 3 cashiers or hostesses and one manager. Eight of the employees interviewed reported illness during the outbreak period July 9 to July 27, and four of them worked while symptomatic (**appendix S, Figure 5**). Six (75%) of these employees submitted stool specimens.

On July 16, the day the two customers who acquired *E. coli* O157:H7 infections ate at the MRSR, 18 employees were working. Ten of those working on that day submitted stool specimens and two reported illness during the outbreak period (**appendix S**). Stool specimens submitted by all 24 of the restaurant employees were negative for enteric pathogens (**appendix N**).

During the MRSR employee interviews, there were many discrepancies in the answers provided (**appendix T**). For example, employees provided different answers about hand washing training, filling in for employees at the other Sizzler restaurant, location of thermometers to check food temperatures, who checks food temperatures, safe temperatures for hot and cold foods, and the procedure for handling dirty dishes. While no unifying cause of the outbreak was identified from the interviews, several practices were reported that were possible mechanisms of transmission of infectious pathogens. Some of the reported practices were environmental practices that could increase the risk of cross-contamination of food. The lettuce shredder was reportedly stored in the meat prep room under the soup containers. There were also reports of equipment such as a slicer being used to cut both uncooked meat and ready-to-eat products. The sink used to wash some vegetables was located in the room where meat was tenderized. The grinder that was used to grind meat was also used to whip butter. No signs were posted reminding customers not to reuse dishes.

Other questionable practices within the MRSR were policy-oriented. Hand washing training was reported to be inconsistent. On weekends, it was felt likely that some people would fill in on "whatever job needed to be done", regardless of training in that position. There was no policy assuring that ill workers would not work. Some job positions required handling clean and dirty food. No policy was in place to determine when to dispose of food. Clean dish clothes had to be obtained from the manager. Thermometers were apparently not uniformly available to check temperatures on the hot food bar. Food, including chicken, was reportedly transported from the MRSR to the LASR without refrigeration. Food temperatures were not systematically checked, and proper food temperatures were unknown. Finally, the manager was not a Certified Food Manager as required in s. 254.71(1), Stats.

***Site visits and resulting control measures***

There was no assurance that ill employees refrained from working while ill. At least eight employees reported having signs and symptoms of diarrheal illness during the outbreak period

and four of those employees worked while they were symptomatic. One cook, who did not work while symptomatic, reported symptoms of diarrhea among himself and his three children that ceased just two days before he worked 10 days of a 12-day work period.

Hand-washing facilities were not conveniently located in the kitchen area of the restaurant. The two hand-washing sinks were located in the kitchen and grill areas. One was located at the south end of the grill line and one was located at the north end of the preparation line of counters in the kitchen. There was no hand-washing link in the meat room. There was concern that the sink in the meat preparation room that was for food cleaning might also have been used interchangeably for hand-washing and food cleaning. General knowledge of safe food-handling practices by employees was inadequate.

Meat processing knives were stored in standing water with a sanitizing solution in the meat room. The water was observed as a rust color, possibly the result of meat juices.

Nothing unusual was discovered regarding receipt, storage or handling of chicken wing product. Chicken wings were received from Sysco and immediately stored in a walk-in freezer. Chicken wings were removed from the walk-in freezer to the upright freezer on the cooking line, as needed, and were removed from the upright freezer for frying. Chicken wings were cooked when needed for the buffet and uneaten cooked chicken wings were discarded at the end of the day. Chicken wings were part of the buffet and were not a regular menu item.

Complete reports prepared by the WHD sanitarians **(appendix T)** and another prepared by Division of Public Health, Bureau of Environmental Health (BEH) sanitarians **(appendix U)** are attached. A restaurant floor plan is included as **appendix V.** Considerable time was spent assessing food-handling practices in the facility. Conditions for re-opening were established and presented to the operator/owner on August 16, including a requirement that the operator/owner provide an action plan detailing changes in food-handling practices so as to minimize future risks **(appendix W)**.

## DISCUSSION AND RECOMMENDATIONS

Although the Mayfair Road and Layton Avenue Sizzler restaurant outbreaks occurred in a similar time frame and in geographic proximity, they are distinct outbreaks and have been summarized in separate reports. There is no evidence that any of the cooked or raw products implicated in either outbreak were shared between the two restaurants during the outbreak period. The two outbreaks occurred in separate locations and were caused by different errors in food preparation and handling. The LASR outbreak was caused by cross contamination of watermelon with *E. coli* O157:H7 from the raw sirloin tri tips meat product. It is not unexpected that the 60 *E. coli* O157:H7 human isolates from the LASR outbreak matched the two *E. coli* O157:H7 isolates from the MRSR outbreak pattern by PFGE since both restaurants received and were using the same lot of sirloin tri-tips (XL EST 86R) during the outbreak period. Cross contamination of

different food items at the two restaurants with this same lot number of sirloin tri-tips would result in isolates with the same PFGE pattern.

It is hypothesized that the Mayfair Road Sizzler outbreak was caused by two distinct human pathogens - a Norwalk-like virus and *E. coli* O157:H7. Based on the incubation period, signs and symptoms of MRSR patrons and the case-control statistical analysis, it is most likely that the 19 patrons with shorter incubation illness became ill following consumption of lettuce that was contaminated with Norwalk-like virus. There are many reports in the literature which document the transmission of Norwalk-like viruses from the bare hands of symptomatic food handlers to raw fruits and vegetables.[1-3] There are also reports in the literature which document the continued shedding of Norwalk-like virus in the stool of individuals for periods up to 2 weeks following cessation of diarrhea.[3-5]

The two-laboratory-confirmed cases of *E. coli* O157:H7 infection in the MRSR outbreak appear to be part of a group of 33 patrons with longer incubation illness. The symptom profile, incubation period, and case-control analysis of the 33 longer incubation patrons suggest that their illness was due to *E. coli* O157:H7 infection associated with consuming chicken wings that were cross-contaminated with raw sirloin tri-tip meat product. The mechanism by which this may have occurred is undetermined.

The likelihood of future foodborne outbreaks in similar facilities can be reduced by:

- Providing complete physical separation between meat processing areas and ready-to-eat food preparation areas.
- Ensuring that hand washing facilities are adequately and conveniently located. This cannot be determined by Code provisions alone – in many cases it is necessary to observe actual use (or lack thereof) to determine adequacy.
- Monitoring the knowledge and skill levels of foodservice workers and providing training to ensure that foods are handled safely and that, to reduce the likelihood of cross-contamination, utensils, equipment, and work surfaces are properly cleaned and sanitized between contact with raw food products and ready-to-eat products. Food-handler Certification requirements are a step in the right direction, but how people actually use their knowledge and skills is much more important.
- Ensuring that a HACCP plan or other standard operating procedures are followed.
- Designated cutting boards should be used, cleaned and stored separately. Interchangable use should be discouraged.
- Knives, like cutting boards, and other processing equipment must be cleaned and sanitized after each use. Storage in standing water should be discouraged.

## REFERENCES


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# Investigation of an Outbreak of *E. coli* O157:H7 Infection at the Layton Avenue Sizzler Restaurant, Milwaukee, WI; July – August, 2000

Final Report

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**October 6, 2000**

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*I would like to thank Jeffrey P. Davis, M.D., Chief Medical Officer and State Epidemiologist for Communicable Diseases for critical review of this investigation summary.*

## ABBREVIATIONS

| | |
|---|---|
| BCD | Bureau of Communicable Diseases |
| CDC | Centers for Disease Control and Prevention |
| CDES | Communicable Disease Epidemiology Section |
| *E. coli* | *Escherichia coli* |
| FDA | Food and Drug Administration |
| FSIS | Food Safety and Inspection Service |
| HUS | Hemolytic uremic syndrome |
| LASR | Layton Avenue Sizzler Restaurant |
| MHD | City of Milwaukee Health Department |
| MHDL | Milwaukee Health Department Laboratories |
| PFGE | Pulsed-field gel electrophoresis |
| USDA | United States Department of Agriculture |
| WDATCP | Wisconsin Department of Agriculture, Trade, and Consumer Protection |
| WDPH | Wisconsin Division of Public Health |
| WSLH | Wisconsin State Laboratory of Hygiene |

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## BACKGROUND

On July 24, 2000, the infection control staff at Children's Hospital of Wisconsin, Milwaukee, notified the City of Milwaukee Health Department (MHD) regarding a cluster of cases of *E. coli* O157:H7 infection in children between the ages of four and eight years including one case of hemolytic uremic syndrome (HUS). Completed interviews of the first four children revealed that all recently ate at the Milwaukee area Layton Avenue Sizzler Restaurant (LASR). This information was reported by the MHD to staff at the Wisconsin Division of Public Health (WDPH), Bureau of Communicable Diseases (BCD), Communicable Disease Epidemiology Section (CDES) at 4:39 pm on July 25. That evening the immediate investigation response plan included (1) sending a MHD sanitarian; (2) initiating a line list of laboratory-confirmed *E. coli* O157:H7 infections in the Milwaukee area; and (3) to securing and forwarding the patient isolates to the Wisconsin State Laboratory of Hygiene (WSLH) for pulsed-field gel electrophoresis (PFGE).

## METHODS

**Early control measures:**

Soon after the cluster of cases at Children's Hospital of Wisconsin was linked to the LASR and the restaurant management and owners were made aware of this, they voluntarily closed the restaurant on July 26. Food products were ordered held pending completion of the investigation. As soon as *E. coli* O157:H7 was identified in a meat product taken from the restaurant, the USDA was contacted so that the meat could be traced backed to its source and traced forward to withdraw supplies still in circulation. The MHD, staff at Children's Hospital of Wisconsin, the BCD, the WDATCP, the CDC, USDA, and FDA worked closely and cooperatively during the investigation.

**Surveillance:**

***Case finding***

Press releases On July 26, 2000 the MHD issued a press release **(appendix A)** describing the cluster of cases, explaining how *E. coli* O157:H7 infection is acquired and how to prevent further transmission within the community. The press release detailed where to report suspected cases and how to obtain laboratory confirmation of illness. The press release also requested that persons who ate at the LASR since July 15 call the MHD to be interviewed whether or not they had experienced any signs or symptoms of illness. Press releases were issued by the MHD on an almost daily basis from July 26 through August 30, 2000.

Laboratory surveillance Additional potential cases of *E. coli* O157:H7 infection in the Milwaukee area were identified by review of laboratory reports from the Milwaukee Health Department Laboratories (MHDL), Children's Hospital Laboratory, Medical Sciences Laboratory, St. Francis Hospital Laboratory, St. Mary's Laboratory, Waukesha Memorial Hospital Laboratory, Aurora Clinical Laboratory, and the WSLH.

Listserve posting On July 26 **(appendix B)** and August 2 **(appendix C)** CDES staff posted information regarding the outbreak investigation on CDC's Foodborne Outbreak Listserve to notify CDC and other state health departments and request notification of Wisconsin CDES staff if residents of other states with exposure to the Milwaukee area Sizzler restaurants were discovered.

PulseNet posting On July 28, the WSLH PFGE lab posted the outbreak pattern on CDC's PulseNet system **(appendix D)** to allow other states conducting PFGE on *E. coli* O157:H7 isolates from their state residents to identify specimens with matching PFGE patterns.

***Case definitions***

Potential outbreak-related cases of *E. coli* O157:H7 infection were those which occurred among individuals who ate at the LASR between July 10 and July 21 and experienced signs and symptoms which were consistent with the CDC case definition for *E. coli* O157:H7 infection [Centers for Disease Control and Prevention. Case Definitions for Public Health Surveillance: Infectious Diseases. MMWR 1997;46(RR-10):13-14.] and the Wisconsin Disease Surveillance EPINET Manual **(appendix E)**.

A *confirmed* case was defined as an individual with bloody or watery diarrhea and abdominal cramps or development of hemolytic uremic syndrome (HUS) following diarrheal illness in an individual who ate at the LASR between July 12 and 21. *Laboratory-confirmed* cases included those in which *E. coli* O157:H7 or Shiga toxin-producing *E. coli* was isolated from a stool specimen in an individual who ate at the LASR between July 12 and 21, experienced signs and symptoms consistent with *E. coli* O157:H7 infection. A *probable cases* was defined as bloody or watery diarrhea occurring within 12 days of eating at the LASR between July 12 and 21 in an individual from whom a stool specimen was not obtained for confirmatory testing. A *possible cases* was defined as diarrhea or abdominal cramps or nausea occurring within 12 days of eating at the LASR between July 12 and 21 in an individual from whom a stool specimen was not submitted for confirmatory testing. Primary cases were those which were the first in a given household meeting any of the case definition criteria; secondary cases were those which occurred 3-8 days following onset of a primary case in the same household.

**Epidemiologic surveys:**

***Case control study***

An outbreak-specific case-control interview form **(appendix F)** was developed by CDES staff which included demographic data, information about signs and symptoms of disease, consumption of 140 food items listed on the LASR menu and questions regarding other known risk exposures for *E. coli* O157:H7 infection. Laboratory-confirmed case-patients were interviewed using this outbreak-specific case-control questionnaire. Community and family controls who ate at the Sizzler restaurant between July 12 and 21 but who did not experience signs or symptoms of illness were interviewed using the same case-control questionnaire used to interview laboratory-confirmed case-patients.

**Laboratory Investigation:**

***Bacteriologic and molecular analysis of human, environmental, and food specimens***
Milwaukee Health Department Laboratories The MHDL tested Layton Avenue employee stools, environmental swabs from the kitchen food prep area, and food samples from the food buffet lines at the time of MHD restaurant inspections. The MHDL also tested unopened packages of food items in the refrigerator and freezer collected during a series of restaurant inspections during the outbreak inspection.

Stool specimen kits were supplied by the MHD to individuals who did not have a primary care physician who could provide testing. Stool specimens were screened for *E. coli* O157:H7 using MacConkey sorbitol medium (Difco Laboratories, Detroit, MI) and sorbitol negative colonies were confirmed as *E. coli* O157:H7 using O157 and H7 agglutination tests. Individual case-patient and food sample *E. coli* O157:H7 isolates were forwarded to the WSLH for PFGE analysis.

United States Department of Agriculture
The USDA inspector picked up samples from the LASR on August 1 and August 2 and forwarded them to the USDA laboratory in Athens, GA for pathogen analysis.

***Pulsed field gel electrophoresis***
The PFGE method was conducted by standard technique (Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention. PulseNet – The national molecular subtyping network for foodborne and disease surveillance. One day standard laboratory protocol for molecular subtyping of *Escherichia coli* O157:H7 by pulsed-field gel electrophoresis, 1998). Genomic DNA in agarose plugs was restricted with XbaI and BlnI as recommended by CDC's PulseNet procedure. The resulting macrorestriction fragments were separated by CHEF-PFGE using a CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, CA) at 200 V and 14$^0$C for 18 to 22 hours with switching times ranging from 2.16 to 54.17 seconds. E. coli G5244 was used as the reference standard. Molecular Analyst-Finger Printing Plus software (Bio-Rad) was used for analysis of restriction bands with normalization to the PulseNet Global Standard provided by CDC.

**Environmental Investigation :**

***Restaurant inspection and sample collection***
The LASR was inspected the evening of July 25 and food samples were collected from the buffet lines and walk-in cooler and submitted to the MHDL for pathogen analysis. The restaurant management team and members of MHD met several times during the next week at the restaurant to review food preparation processes and procedures, and to look at the physical layout of the establishment. Invoices of food purchases between July 3 and July 21 were obtained to facilitate any traceback of food that would be necessary. Inspectors from the MHD, WDATCP,

USDA, and FDA visited the restaurant during the mornings of August 1 and 2 when more samples were collected and forwarded to the MHDL as well as USDA laboratories. The MHD worked cooperatively with the WDATCP, USDA, and FDA to identify the source of the contaminated meat.

***Employee interviews and specimen collection***

Between July 26 and August 22, 2000, LASR employees were interviewed, asked about food preparation methods, and requested to submit a stool specimen for pathogen analysis. The LASR employs 35 full or part-time employees including 14 servers, 4 utility workers (dishwashers), 7 salad bar workers, 3 cooks, 5 cashiers or hostesses and 2 managers. Stool specimen kits were provided by the MHD to employees of the LASR and forwarded to the MHDL for pathogen screening.

**Data analysis:**

Analysis of case-control data was done using Mantel-Hanszel matched chi-square analysis for dichotomous variables using Epi-Info (version 6.0; CDC, Atlanta, GA). Ninety-five percent confidence intervals for odds ratios were calculated using the method of Cornfield; probability values (p value) were calculated by Fishers' 2-tailed exact test. Multivariate analysis was performed using Statistics® analytic software (Tallahassee, FL) and was based on Mantel-Hanszel methodology.

## RESULTS

**Surveillance:**

***Case finding***

Sixty-two *confirmed* cases (60 *laboratory-confirmed* cases with an *E. coli* O157:H7 isolate, one individual with HUS case without an isolate, and one individual with a Shiga toxin-producing *E. coli* isolate) were identified as associated with eating at the LASR during July 12 to 21, 2000 **(appendix G, Figure 1).** Forty-four (71%) of the cases were females and 18 (29%) were male. Laboratory-confirmed cases were reported among residents of 4 Wisconsin counties (Milwaukee, Kenosha, Racine, and Sauk) and one resident each of France and the states of Illinois, New York, and Washington who had been visiting the area when the outbreak occurred. Among approximately 1700 calls received by the MHD, 551 probable cases (gastrointestinal signs and symptoms with bloody or watery diarrhea) and 122 possible cases (gastrointestinal signs and symptoms) were also identified in Milwaukee area residents who called the MHD to be interviewed in response to the press releases.

***Descriptive epidemiologic features***

The mean age of the 62 confirmed case-patients was 28.5 years (median, 15.0; range 1 to 86). Twenty-three (37%) case-patients were hospitalized including four (6.4%) with hemolytic uremic syndrome (HUS). One individual with HUS died. The first confirmed case experienced onset of signs and symptoms on July 14 and the last experienced illness onset on August 8 **(appendix H,**

**Figure 2)**. The most frequently reported signs and symptoms included diarrhea (97%), fatigue (79%), abdominal cramps (76%), and nausea (69%) **(appendix I, Table 1).** Mean incubation period was 4.04 days (median 3.0; range 2 to 24 days) and duration of diarrhea was 6.4 days (median 6.5 days; range 2 to 11 days).

**Analytic epidemiologic findings:**

***Case-control study***

Analysis of case-control interview data using the LASR menu that included over 140 food items identified watermelon as the only food statistically associated with illness (odds ratio = 8.83; 95% confidence interval = 3.1 to 26.2, p=0.0000056). The full attack rate table for the case control study at the LASR is included as **appendix J.** The epidemiologic study did not link illness with eating at other restaurants or with other known risk factors associated with previously reported outbreaks of *E. coli* O157:H7 including consuming unpasteurized milk, contact with cattle or cattle manure, recreational water exposure, diaper changing, or contact with an individual with known *E. coli* O157:H7 infection.

**Laboratory investigation:**

***Bacteriologic and molecular testing of human specimens***

Case-patients All 60 *E. coli* O157:H7 case-patient isolates were indistinguishable from each other by PFGE using both Xba1 and Bln1 enzymes. In addition, 26 other *E. coli* O157:H7 specimens received at the MHDL and WSLH during the outbreak period from individuals who had not eaten at the LASR had PFGE patterns which were different from each other and from the Sizzler outbreak pattern. A dendrogram detailing PFGE of 51 of the outbreak-related *E. coli* O157:H7 isolates and 6 of the non-outbreak isolates is included as **appendix K.**

Restaurant employees

Twenty-seven of the 34 LASR employees (79%) submitted stool samples for culture. The culture of one employee, a wait staff, was culture-positive for *E. coli* O157:H7 with a PFGE pattern matching the outbreak pattern. This stool sample was collected on July 29, 2000. Follow-up stool samples collected from this individual on August 8 and 19, 2000 were negative for *E. coli* O157:H7. This wait staff who denied eating anything at the restaurant while at work except for oyster crackers was asymptomatic and worked on July 7, 14 and 21, 2000. The remainder of the employee stool samples were negative for *E. coli* O157:H7.

***Testing of environmental and food samples***

Milwaukee Health Department Laboratories During the investigation into the source of *E. coli* O157:H7 contamination at the Layton Avenue Sizzler location, a total of 106 samples were obtained from the restaurant. Ninety-two samples were food items remaining in the restaurant and the remaining 14 samples were obtained from the environment surrounding the food items and kitchen area. Thirty-seven percent (34/92) of the food items collected from the restaurant were cultured for pathogens. Among the food items cultured, three resulted in positive *E. coli*

O157:H7 results: two from beef patties served as a children's entrée and one from the ground taco meat served at the taco bar. None of the 14 environmental restaurant samples was positive for *E. coli* O157:H7 **(appendix L).**

The beef patties were received by the restaurant from a distributor as preformed frozen patties and were stored in a "walk-in" freezer. Of the two beef patties tested, one was frozen and the other had been thawed in preparation for cooking. Both beef patties initially tested positive for *E. coli* O157:H7 but were negative upon confirmation testing.

The taco meat is prepared by grinding up chunks of sirloin that are trimmed off sirloin tri-tips roasts. Both initial and confirmatory tests of the taco meat were positive for *E. coli* O157:H7. PFGE molecular fingerprinting confirmed that the *E. coli* O157:H7 isolate from the taco meat was indistinguishable from the PFGE pattern of the *E. coli* O157:H7 isolates from the 62 human laboratory-confirmed cases **(appendix M).**

United States Department of Agriculture (UDSA) Traceback of information from LASR and Mayfair Road Sizzler Restaurant invoices for the month of July indicated that both restaurants had obtained raw meat products from Excel Corporation, Wichita, Kansas, during the outbreak period. The USDA secured and tested a total of 22 meat products associated with lot numbers shipped to the restaurants during the outbreak period: 8 were obtained from the FSIS plant, 10 were collected at Wisconsin state plants, and 4 were Sizzler restaurant samples. None of the 22 specimens tested by the USDA laboratory was positive for *E. coli* O157:H7 **(appendix N).** An intact cryovac sirloin tri-tips roast (with the same lot # XL EST 86R as invoices indicated were at the LASR and the Mayfair Road Sizzler restaurant at the time of the outbreak) was obtained by USDA staff from the LASR walk-in cooler on August 1 and forwarded to Summerfry Labs (SF) for pathogen isolation. SF labs identified two *E. coli* O157:H7 isolates from this intact sirloin tri-tip sample **(appendix N).** These two *E. coli* O157:H7 isolates from the intact sirloin tri-tips (BC 5674 & BC 5677) were forwarded to the WSLH where the PFGE pattern was found to be indistinguishable from the *E. coli* O157:H7 isolate from the raw chunky taco meat from the LASR (BC 5033), the LASR outbreak-related patient isolate pattern (BC5028 &BC5029), and the Mayfair Road Sizzler restaurant patient isolate pattern (BC 5131) **(appendix O).**

Food and Drug Administration After the association of illness with watermelons was identified in the case-control study, intact watermelons in the LASR walk-in cooler were tested by the MHDL using an FDA method provided by Sherry McGarry from the CFSAN outbreak investigation team for detection of *E. coli* O157:H7 on the rind and interior of melons. Samples from one uncut watermelon were shared with FDA and Summer Fry laboratories. The screening test for all watermelons was negative at the MHDL **(appendix P)** and at FDA (personal communication, Clifford Purdy, FDA liaison to CDC).

**Environmental investigation**:

***Site visits and resulting control measures***

During the inspection of the LASR on the evening of July 25, food samples were collected from the buffet lines and walk-in cooler. Three violations were noted: the dishwasher was operating at a low temperature and was out of chemical sanitizer; the dishwasher did not contain an automatic signaling device to indicate when sanitizer was low or out; and several bowls of various melons were measured to be 48°F through 53°F on the buffet line. In addition, during the inspection it was learned that most food items on the buffet were refrigerated and reused, including items from the kid's buffet and that an ill child of a worker, still in diapers, had an *E. coli* O157:H7 infection.

Inspectors visited the restaurant again on the morning of July 26 and more samples were obtained for testing. The restaurant voluntarily closed on July 26 as the number of *E. coli* cases continued to increase. The restaurant management team and members of the MHD met several times during the next week at the restaurant to review food preparation processes and procedures and to look at the physical layout of the facility.

Considerable time was spent assessing food-handling practices in the facility. Conditions for re-opening were established and presented to the operator/owner, including a requirement that the operator/owner provide an action plan detailing changes in food-handling practices to minimize future risks.

***Sanitarian report***

The Sizzler is a medium-sized, full-service restaurant serving both lunch and dinner with an average of 300 meals served per day. The operator of the Layton Avenue facility also owns and operates another Sizzler on Mayfair Road in Wauwatosa, WI. At approximately the same time period as the LASR outbreak, another outbreak was occurring at the Mayfair Sizzler in Wauwatosa. Two laboratory-confirmed cases of *E. coli* O157:H7 infection occurred in individuals who ate only at the Mayfair Sizzler and these two isolates were indistinguishable by PFGE from the 62 *E. coli* O157:H7 isolates from the LASR outbreak. The Wauwatosa Sizzler outbreak was not caused by cross contamination of watermelon with sirloin tri-tips but was caused by cross contamination of multiple salad bar items with raw meat. Therefore, although the two outbreaks at two Sizzler restaurants occurred in roughly the same time-frame, they are considered different outbreaks and have been summarized separately.

The Sizzler restaurants served a diverse population which included families with young children and the elderly. The restaurant offered a conventional menu consisting primarily of grill items (hamburger, seafood, steaks, and chicken entrées) and an extensive salad bar. Foods on the salad bar included cold salad components, soups, hot appetizers, taco ingredients, fruit and prepared salads. There was also a dessert bar and a "kid's bar". Foods were either prepared from scratch or purchased pre-cooked and reheated.

The restaurant employed a diverse multi-lingual workforce of approximately 35 people, the majority of whom worked part-time. Communication barriers existed which may have prevented employees from learning and properly performing their job duties. Employees' experience levels ranged from very little (less than 3 months with no food service background) to extensive (over 5 years). Although employees had specific job descriptions, their duties varied considerably depending on staffing levels. For instance, cashiers and others did salad preparation work between July 8 and July 16 when the primary salad person was on vacation.

The "back of the house" facilities consisted of a front cook line and grill area, a food preparation area, a meat processing area, a dishwashing room, a waitress area and storage areas. A pre-wash/hand-washing sink was located in the dishwashing area and one additional hand-washing facility served the remaining areas. There were no hand-washing facilities in the front cook line area.

Most food preparation for the salad bar took place on two tables located side-by-side, with a stand mixer between them. The mixer was equipped with a removable grinder attachment and was used to whip butter, make frostings, seafood salad, fish batter, and, most significantly, to grind meat. Since both raw meats and foods that would not receive heat treatment prior to consumption were prepared in such close proximity, the potential for cross-contamination existed.

Except for meat products which were obtained from Excel Corporation in Wichata, Kansas, the restaurant purchased most of its food from Sysco, a distributor located in Jackson, WI. Deliveries were made to the LASR twice weekly. The complete sanitarian's report is included in **appendix Q**.

## DISCUSSION AND RECOMMENDATIONS

The layout of the facility and the practices of personnel may have contributed to this outbreak. The arrangement of a meat processing area (the grinding area) in close proximity to ready-to-eat food preparation areas increased the likelihood of cross-contamination. Although there was a separate meat processing area where most cutting and other processing was done, the grinding of meat was done in an area where ready-to-eat foods were prepared.

There was no assurance that ill employees refrained from food-handling activities. A cook reported working with diarrhea, stomach pains, and nausea for eight days just prior to the outbreak. He felt that he would lose his job if he failed to report for work due to illness. A cashier with similar symptoms also worked while ill during this period, and her child, still in diapers, was laboratory-confirmed for *E. coli* O157:H7 early in the outbreak. This child frequently spent time and consumed food at the restaurant between shifts. Cashiers' activities included some food preparation. In addition, one employee had laboratory-confirmed *E. coli* O157:H7 infection during this time period, but reported being asymptomatic.

Hand-washing facilities were not conveniently located. Only one hand-washing sink was available for the food preparation area and front cook line area, and several cooks admitted that they "washed" their hands in sanitizer buckets.

Methods used to replenish the salad bar may have also contributed to this outbreak. Food items were rotated – old food was put on top of new food in a continual cycle. If ready-to-eat food items, such as watermelon, were to become contaminated via a cutting board, for example, this rotation method could allow contaminated juices to flow into fresh foods, continually re-contaminating the food. This would explain the prolonged ten-day exposure period (July 12 to July 21) during which restaurant patrons became ill. An initial single episode of cross contamination could have been propagated through the rotational method of replenishing the salad bar.

General knowledge of safe food-handling practices by employees was inadequate. For example, several cooks stated that they used thermometers and took temperatures, yet none of the cooks interviewed knew the safe cold food temperature and only one knew the safe-re-heating temperature.

Based on the results of the case-control study, the test results of the opened and intact food samples from the restaurant and the conclusions of the restaurants inspections, it is most probable that the watermelon was the vehicle for infection, cross-contamination of fresh watermelon with raw meat product was the mechanism by which the vehicle became contaminated, and the raw sirloin tri-tips were the source of *E. coli* O157:H7 organisms in this outbreak.

The likelihood of future foodborne outbreaks in similar facilities can be reduced by:

- Providing complete physical separation between meat processing areas and ready-to-eat food preparation areas.
- Ensuring that hand washing facilities are adequately and conveniently located. This cannot be determined by Code provisions alone – in many cases it is necessary to observe actual use (or lack thereof) to determine adequacy.
- At facilities using salad bars, ensuring that old food is not indefinitely recycled by mixing it with new food.
- Ensuring that all potentially hazardous foods are held at safe temperatures.
- Monitoring the knowledge and skill levels of foodservice workers and providing training to ensure that foods are handled safely and that, to reduce the likelihood of cross-contamination, utensils, equipment, and work surfaces are properly cleaned and sanitized between contact with raw food products and ready-to-eat products. Food-handler Certification requirements are a step in the right direction, but how people actually use their knowledge and skills is much more important.
- Ensuring that a HACCP plan or other standard operating procedures are followed.

# 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 beef[1-4] 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.

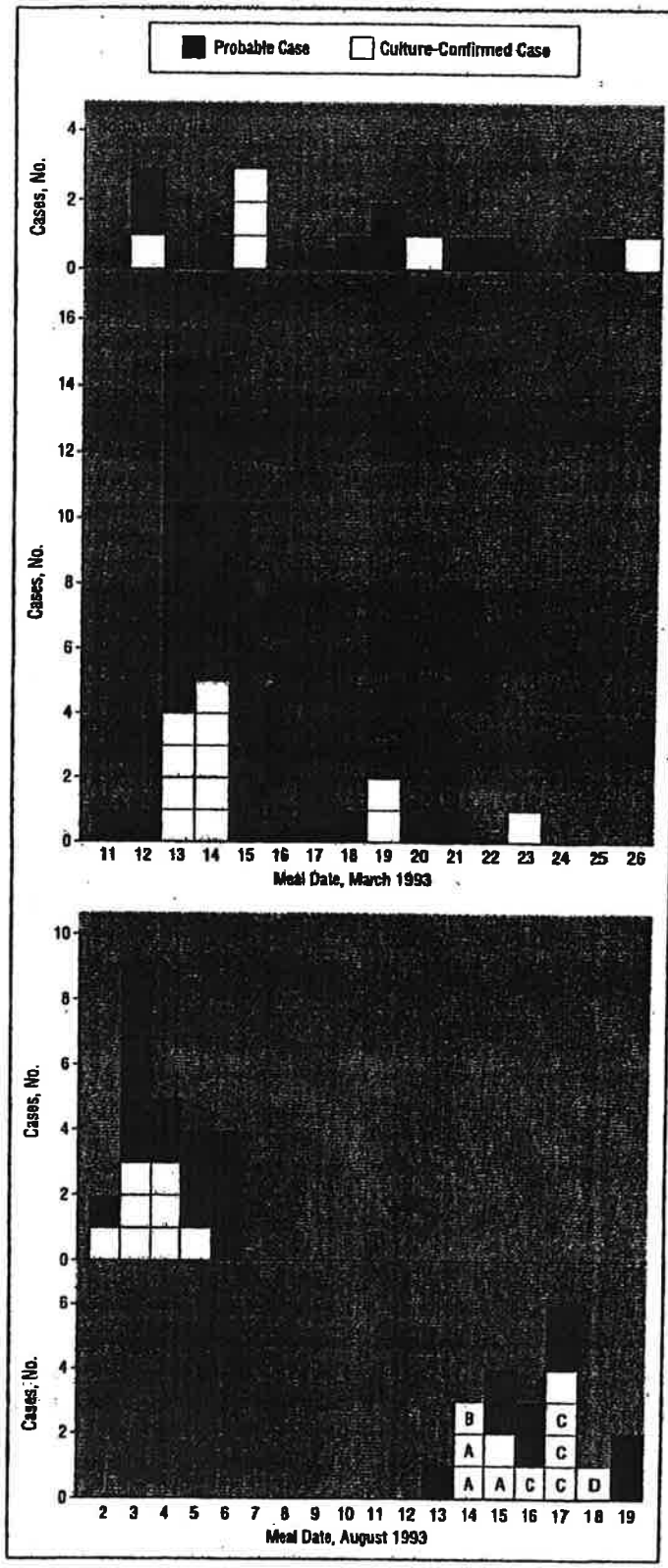
Differences in proportions were assessed using the $\chi^2$ statistic or the Fisher exact test when an expected cell size was less than 5.

### 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 described[15] 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]

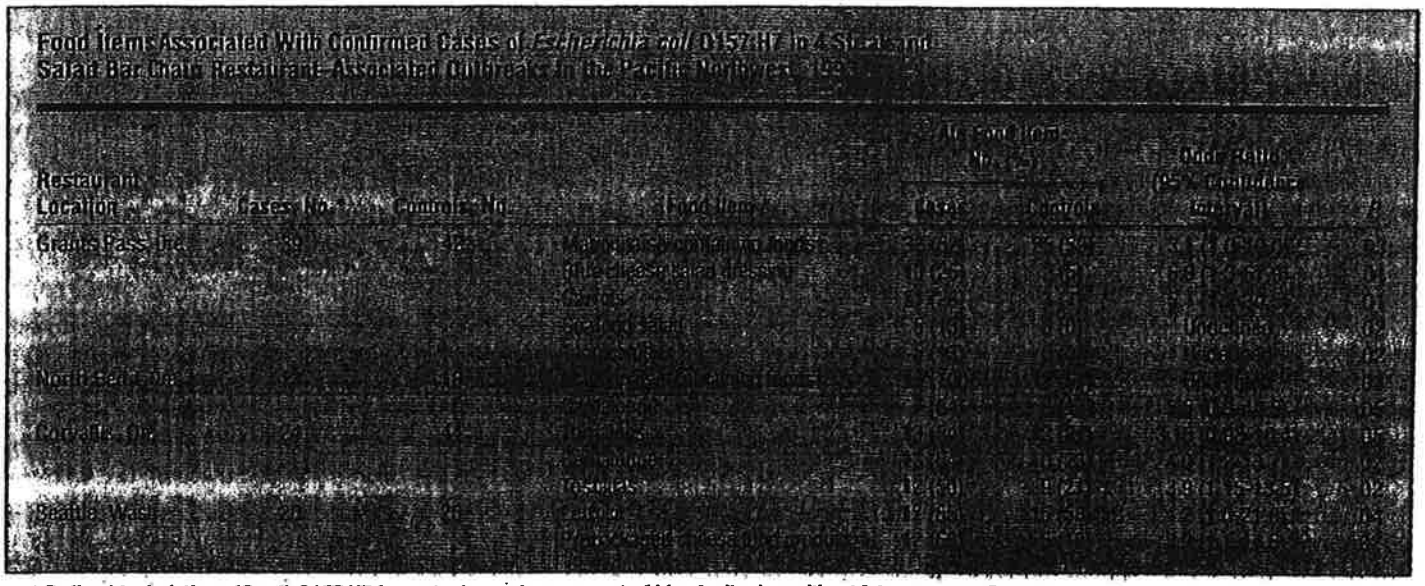



*Reported meal dates for* Escherichia coli *O157:H7 cases associated with outbreaks at 4 chain Z restaurants in 1993. Letters indicate the pulse-field gel electrophoresis subtypes of case isolates from the Seattle, Wash, outbreak.*

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.

## 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



*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-RFLP[17] 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 vaccines[33] 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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