

A Multistate Outbreak of *Escherichia coli* O157:H7 Infection Linked to Consumption of Beef Tacos at a Fast-Food Restaurant Chain

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(See the editorial commentary by Osterholm on pages 8–10)

We investigated a multistate outbreak of *Escherichia coli* O157:H7 infections. Isolates from 13 case patients from California, Nevada, and Arizona were matched by pulsed-field gel electrophoresis subtyping. Five case patients (38%) were hospitalized, and 3 (23%) developed hemolytic uremic syndrome; none died. The median age was 12 years (range, 2–75 years), and 10 (77%) were female. Case-control studies found an association between illness and eating beef tacos at a national Mexican-style fast-food restaurant chain (88% of cases versus 38% of controls; matched OR, undefined; 95% confidence interval, 1.49 to infinity; $P = .009$). A trace-back investigation implicated an upstream supplier of beef, but a farm investigation was not possible. This outbreak illustrates the value of employing hospital laboratory-based surveillance to detect local clusters of infections and the effectiveness of using molecular subtyping to identify geographically dispersed outbreaks. The outbreak investigation also highlights the need for a more efficient tracking system for food products.

First recognized in 1982, *Escherichia coli* O157:H7 infection is now a major cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS) [1, 2]. Foodborne transmission is believed to account for 85% of the 73,000 estimated cases of *E. coli* O157:H7 infection per year in the United States [3]. In 1993, awareness of the threat from *E. coli* O157:H7 infections was enhanced when a multistate outbreak of infection involving 700 illnesses and 4 deaths was associated with eating hamburgers at a national fast-food restaurant chain in the United States [4]. In recent years, outbreaks of *E. coli* O157:H7 infections have been caused by contaminated produce and juices; however, undercooked ground beef

remains the most common vehicle of transmission [5–7]. Cattle are known reservoirs of *E. coli* O157:H7, which colonizes the bovine intestinal tract asymptotically [8–11]. Contamination of beef products primarily occurs during slaughter when meat is contaminated by fecal material. It has been reported that *E. coli* O157:H7 is prevalent among cattle entering the slaughterhouse and cattle carcasses at all stages of the slaughter process [12].

Outbreaks of infection traced to fast-food hamburgers have been uncommon since 1993, possibly because of standardization of cooking processes for ground beef. The present article describes epidemiologic and trace-back investigations that followed the identification of a multistate *E. coli* O157:H7 outbreak linked to a national, Mexican-style, fast-food restaurant chain. The outbreak highlights improvements in our surveillance system, especially timely recognition of outbreaks through molecular surveillance techniques, but it also illustrates significant challenges for the fast-food industry and the limitations of current trace-back investigations of foodborne illness.

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OUTBREAK SUMMARY

Valley Children's Hospital (now Children's Hospital Central California) in Madera, California, began routinely screening for *E. coli* O157 in children with unexplained diarrhea in the fall of 1999 and subsequently discovered 5 cases of *E. coli* O157 infection during the first 2 weeks of November 1999. Interviews by public health staff of the Fresno County Health Department (Fresno, CA) revealed that all case patients had eaten at a popular fast-food restaurant chain (chain A) in the 7-day period before the onset of illness. The infection cluster was reported to the California Department of Health Services (CDHS).

SUBJECTS AND METHODS

Epidemiologic Investigation

Local health officials and clinicians throughout California were asked to enhance surveillance for *E. coli* O157 infections. The Centers for Disease Control and Prevention (CDC) alerted states bordering California and requested that they review medical histories of persons with recent *E. coli* O157 infections and arrange for PFGE subtyping of recent *E. coli* O157 isolates. To identify risk factors for infection, 2 sequential case-control studies were conducted in early December 1999.

Case-control study I. We conducted a case-control study to determine the restaurant associated with the outbreak. For this study, a "case" was defined as a patient with (1) an infection with the PFGE-defined "outbreak strain" of *E. coli* O157:H7, a clinically compatible illness defined as a diarrheal illness with ≥ 3 loose stools during a 24-h period, and/or an HUS during the first 2 weeks of November 1999; or (2) an illness clinically compatible with *E. coli* O157:H7 infection, without laboratory confirmation but with epidemiologic connection to the outbreak. A "control" was defined as a person without a diarrheal illness or HUS during the first 2 weeks of November 1999. Controls were age-matched and systematically identified using computer-assisted telephone interviewing of residents in the same telephone exchange area as case patients. We endeavored to obtain 2 controls per case. Case patients and controls were queried using a standardized questionnaire to determine whether they had eaten at a number of national fast-food restaurant chains in the week before illness onset. Interviews were completed during the first week of December.

Case-control study II. On the basis of results of the case-control study and laboratory testing, a second case-control study involving patrons of chain A restaurants was conducted to determine the specific menu item or ingredient associated with illness. For this study, a case was defined as above but restricted to those who had eaten at chain A, and only case patients that could be matched with "meal companion-controls" were selected. Cases and controls were asked about con-

sumption of specific foods and beverages that appeared on the chain A restaurant menu.

Statistical Analysis

Data were analyzed using EpiInfo, version 6.0 (CDC), and SAS, version 8.0 (SAS Institute). When possible, we calculated Mantel-Haenszel matched ORs (mORs), exact 95% CIs for the maximum likelihood estimate (MLE) of the OR, and corrected Mantel-Haenszel summary χ^2 *P* values using EpiInfo, version 6.0. If the OR or 95% CI could not be calculated using EpiInfo because there were 0 cells, then the conditional MLE of the OR and the mid-*P* 95% CIs for the conditional MLE of the OR were calculated using Exact software, version 0.3 [13].

Laboratory Analysis

Fecal specimens obtained from cases were plated on sorbitol-MacConkey agar. Isolates of *E. coli* O157:H7 were confirmed serologically and were subtyped using PFGE. PFGE was performed using digestion with *Xba*I, followed by digestion with *Bln*I, according to the PulseNet standardized protocol [14]. PFGE patterns were compared with those of historical isolates and considered to be indistinguishable if ≤ 1 band difference was detected after both digestions. Data on the outbreak strain were posted on PulseNet, CDC's national molecular subtyping network for foodborne disease surveillance [14].

Additional tests were performed to determine whether the outbreak strain had any unusual characteristics that could explain survival after having been cooked for adequate times at adequate temperatures. Five *E. coli* O157:H7 strains isolated from cases associated with the outbreak in California were compared with *E. coli* O157:H7 and non-O157:H7 strains isolated from cattle feces [15]. Heat resistance was determined by testing the survival rate of bacteria at a concentration of 5×10^8 cfu/mL in phosphate-buffered saline incubated in a water bath for 5, 15, 40 and 60 min at 55°C and 75°C. After incubation, samples were diluted in Luria-Bertani (LB) or M9 broth, dilutions were plated on the corresponding LB or M9 agar, plates were incubated overnight, and colony-forming units were counted.

Trace-Back Investigation

CDHS, CDC, the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), and corporate management from chain A traced meal ingredients common to all the food consumed by case patients.

Environmental Investigation

Local environmental health inspectors conducted inspections of implicated chain A restaurants in California, Arizona, and Nevada. CDHS's Food and Drug Branch reviewed recent environmental health inspection and complaint reports from all

chain A restaurants in northern California. FSIS obtained records from the supplier of precooked beef and inspected the implicated distributors and processors.

RESULTS

Epidemiologic Investigation

Thirteen case patients were identified from California (10 patients), Nevada (2), and Arizona (1) with illness onsets during 5–17 November. Five cases (38%) were hospitalized, and 3 (23%) developed HUS; none died. The median age was 12 years (range, 2–75 years), and 10 cases (77%) were female.

Case-control study I. We enrolled 10 cases and 19 matched controls from California in case-control study I. Of the 9 restaurants, only chain A showed a statistically significant association with illness among cases and controls (90% vs. 26%; mOR, undefined; 95% CI, 2.33 to infinity; $P = .002$).

Case-control study II. We enrolled 8 cases and 16 meal companion–controls from California, Nevada, and Arizona in case-control study II. Seven cases (88%) ate a beef taco consisting of a hard tortilla shell, ground beef, lettuce, and cheddar cheese, compared with 6 matched meal companions (38%). Consumption of a beef taco was statistically associated with illness by univariate analysis (mOR, undefined; 95% CI, 1.49 to infinity; $P = .009$) and multivariate analysis (mOR, 6.6; 95% CI, 1.1–38; $P = .04$).

Laboratory Analysis

All 13 outbreak-related *E. coli* O157:H7 isolates from the case patients had indistinguishable PFGE patterns after digestion with 2 enzymes, representing a unique PFGE pattern in the PulseNet database. A review of other *E. coli* O157:H7 strains posted on PulseNet during the study period revealed a PFGE match between our isolates and those from a concurrent outbreak investigated in Idaho. The Idaho outbreak involved 20 confirmed and 13 probable cases of infection with illness onsets between 16 October and 18 November 1999. The outbreak in Idaho was independently investigated and linked to wild-game pepperoni purchased from a custom meat processor in Idaho, as shown in figure 1 [16]. *E. coli* O157:H7 was subsequently isolated from leftover wild-game pepperoni and found to have the same unique PFGE pattern as isolates from the patients in the Idaho outbreak and the cases in our study. We were unable to compare findings from lots of ground beef supplied to chain A restaurants with “use by” dates coinciding with our outbreak, because all representative samples had been consumed. The isolates recovered from individuals in California were within normal limits with regard to overall heat resistance.

Trace-Back Investigation

Ninety-two percent of cases ate at 11 different chain A restaurants (figure 1). The ingredients in the beef tacos from asso-

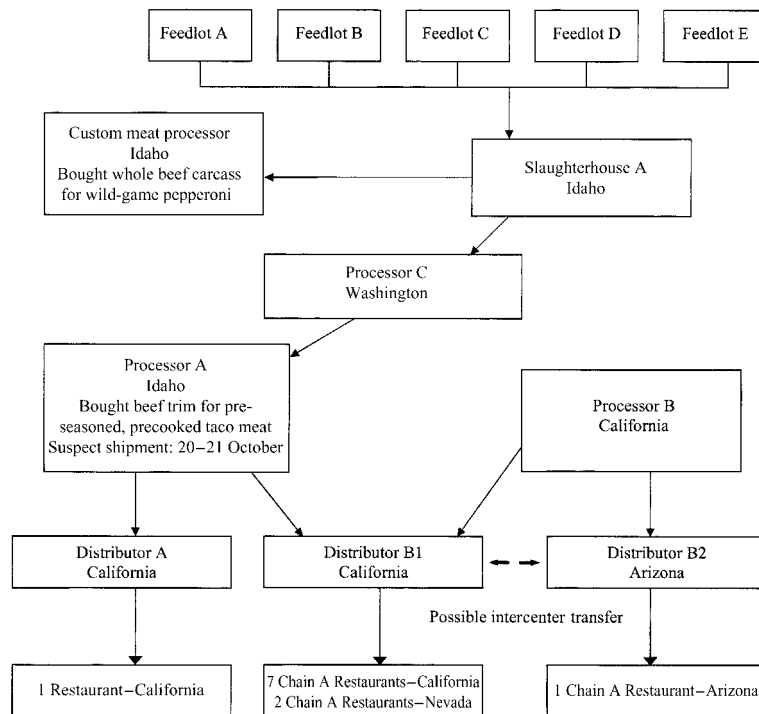


Figure 1. Flow diagram of production and distribution of beef products for chain A restaurants in Arizona, California, and Nevada and a custom meat processor in Idaho.

ciated chain A restaurants were uniform and consisted of pre-cooked and preseasoned ground beef, prepackaged and prewashed shredded lettuce, and pasteurized, shredded cheddar cheese in a precooked hard-shell tortilla. The products were distributed to the implicated restaurants via several distributors in California and Arizona (figure 1). One restaurant in California received food products from distributor A. The other 7 restaurants in California and the 2 restaurants in Nevada received food products from distributor B1. The single restaurant in Arizona received foods from distributor B2. Chain A restaurants did not have the authority to purchase taco ingredients from alternative distributors, and no such transfers were documented. Processors based in California supplied the cheddar cheese and lettuce. These processors supplied cheddar cheese and lettuce to many other fast-food restaurant distributors in which no other illnesses were identified during the outbreak period, including chain restaurants not implicated in case-control study I.

Ground beef was supplied by 2 ground beef processors, 1 based in western Idaho (processor A) and 1 in southern California (processor B). Distributor A received direct shipment of product from the beef processor in Idaho only. Distributor B1 received direct shipment of product from both processors, whereas distributor B2 received direct shipment of product from the California processor only. Distributor B2 intermittently received

ground beef from distributor B1 through the practice of “inter-center transfer,” which allows distributors to transfer ground beef to each other as needed (figure 1). Ground beef from the Idaho facility could have been received by distributor B2 in this manner; however, no documented transfer during the Arizona case patient’s exposure period was identified.

On the basis of available distribution records, 12 (92%) of 13 cases were most likely exposed to beef that was processed at processor A on 20–21 October (figure 2). We traced beef trim included as an ingredient in the ground beef to a Washington processor (processor C) and, from there, to slaughterhouse A in Idaho. A trace-back investigation of the concurrent outbreak in Idaho revealed that whole beef carcass from slaughterhouse A was also an ingredient in the wild-game pepperoni product implicated in the Idaho outbreak (figure 1). According to documentation at slaughterhouse A, the beef sold to processor A and the custom processor of the wild-game pepperoni originated from 5 feedlots (feedlots A, B, C, D, and E). However, the carcasses sent to the processor of wild game were of animals slaughtered days before those that were slaughtered and sent to processor A. A trace-back investigation performed to the farm level for animals in the implicated feedlots was impossible, because beef carcass records were not maintained for the interval between slaughter and processing.

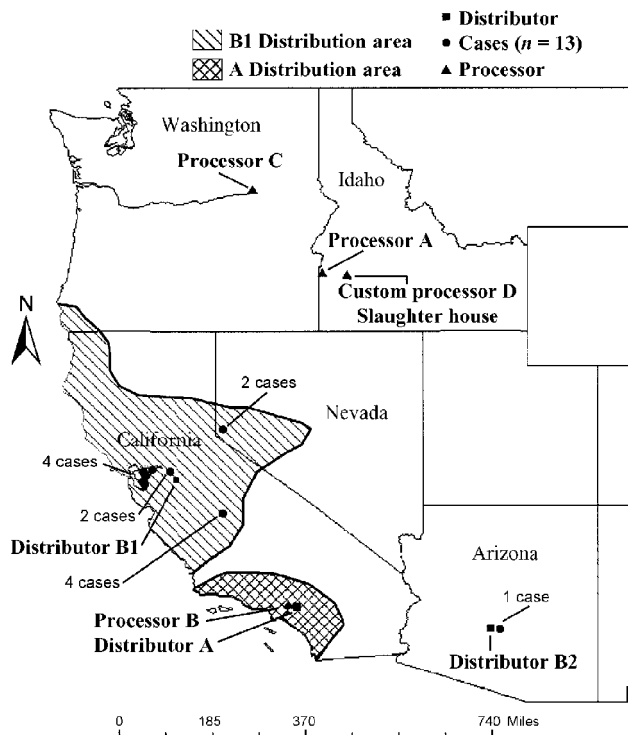


Figure 2. The distribution of ground beef to chain A restaurants, and the location of associated *Escherichia coli* O157:H7 infections in the western United States, November 1999.

Environmental Investigation

Inspection of the chain A restaurant in Nevada revealed time-temperature abuse, and environmental health inspectors had reported previous food safety problems at this restaurant. Our inspection of the other 9 implicated restaurants and the meat processors revealed modern facilities with no obvious system failures that could have accounted for contamination or insufficient cooking of the ground beef. An on-site investigation of the slaughterhouse was not performed.

The restaurant chain participated in a Hazard Analysis Critical Control Point (HACCP) program that included 2 critical steps to kill pathogenic bacteria in ground beef before consumption by the customer. The processors used a patented processing formula in which the ground beef was seasoned and precooked to a target temperature of 82°C for 55–60 min. The taco meat was packaged in 2.3-kg sealed bags and frozen before shipment to the distributors. At the restaurant, the bag of taco meat was reheated to 74°C for 30 min. The bag was removed from the reheating tub and placed in a heated cabinet with a temperature of 75°C. Later, the meat was transferred to a heated holding table from which it was served; holding temperatures for the tables were maintained at 88–93°C.

DISCUSSION

This multistate outbreak of *E. coli* O157:H7 infection was discovered because of traditional public health and laboratory surveillance combined with state-of-the-art molecular surveillance. Implementation of routine screening of all patients with undiagnosed diarrhea for *E. coli* O157:H7 using sorbitol-MacConkey plating media at a children's hospital in Madera was the key to the timely recognition of the first cases. Routine use of sorbitol-MacConkey agar to diagnose *E. coli* O157 infection in persons with diarrheal illness has been recommended [17–19]. Screening is especially important in children's hospitals, because children are more susceptible to severe illness.

The outbreak also illustrates the important role of molecular subtyping surveillance, when integrated with traditional laboratory surveillance. In recent years, PFGE subtyping of *E. coli* O157 isolates has changed from a mostly academic endeavor (often done after an outbreak) to a dynamic, real-time tool for outbreak recognition and investigation [20, 21]. CDC's PulseNet is the national foodborne disease molecular subtyping network of public health and food safety laboratories. In this investigation, posting the outbreak strain's PFGE pattern on PulseNet revealed a link between cases in California, Arizona, Nevada, and Idaho.

Our epidemiologic findings clearly implicated chain A, a national fast-food restaurant chain that serves Mexican-style fast-food. Specifically, consumption of beef tacos in California, Arizona, and Nevada was associated with illness. However, our

epidemiologic analysis could not implicate the specific taco ingredient because of the small number of cases and the uniformity of ingredients in meals eaten by cases and controls. An intensive trace-back investigation of the taco ingredients implicated ground beef, and the source of contaminated beef was strongly suggested, because beef from slaughterhouse A was processed by the meat processor associated with chain A and by the custom meat processor in Idaho (figure 1). In addition, the distribution of case patients more closely matched the distribution of ground beef than it did the distribution of other taco ingredients. An undocumented intercenter transfer of ground beef from distributor B1 to distributor B2 is a possible explanation of the exposure in Arizona. Intercenter transfer of food products between distributors is authorized by distributor B when additional food products are needed to complete an order from one of their restaurant clients. This practice is not common, but we recommend that companies require documentation of all such transfers to improve the traceability of food products.

Chain A restaurants participated in an HACCP program, and no breakdowns were found apart from time and temperature violations at just 1 of the facilities in Nevada. A 2-step heating procedure, first by the processor and then by the restaurant, should have killed any *E. coli* O157:H7 present in the ground beef. Perhaps a limited number of lots or a portion of a lot of contaminated beef was insufficiently cooked by processor A during a brief period. Although records at processor A did not indicate any problems, an undocumented failure during the heating procedure could have occurred. Lapses in the reheating process at just a few restaurants could explain the limited outbreak—for example, problems with reheating procedures were documented at the Nevada restaurant and could have occurred at other restaurants.

We also explored the hypothesis that this outbreak strain could have been unusually resistant to standard heat processing. Testing of the human isolates from this outbreak did not reveal unusual resistance when the isolates were subjected to heat conditions equivalent to those of standard processing. However, the influence of fat content and other characteristics of the finished ground beef product on the survival and growth of these isolates was not examined.

Foodborne disease outbreaks associated with errors in meat processing and/or errors in cooking are well documented [4, 22–24]. Significant advances in food safety have been implemented by the fast-food industry, but complex food distribution channels and frequent employee turnover remain ongoing challenges. This outbreak was particularly frustrating for chain A managers, for public health officials, and for food regulators, because a definitive breakdown in food processing or handling was not identified. The outbreak was characterized by a limited

number of illnesses during a short period, which suggested a brief lapse in safeguards. For these reasons, no specific public health action was taken to control the outbreak, except for recommending continued adherence to HACCP procedures.

This outbreak underscores the need for regulatory agencies and the fast-food industry to improve the traceability of products along the food chain. Canada and the European Union require identification and registration of cattle (with ear tags and computerized data, respectively), which allows trace-back investigations to herds of origin [25, 26]. This identification system in Canada proved to be beneficial after the recent introduction of bovine spongiform encephalopathy (BSE) into the country. Although Canada's animal identification system did not prevent introduction of BSE, it did enable tracking of contact animals [27].

Beginning in July 2004, the USDA Animal Health and Plant Inspection Service will promulgate regulations to implement a US Animal Identification Plan (USAIP) over the next 5 years [28]. The goal of the USAIP is to achieve a trace-back system that will identify all animals and premises potentially exposed to an animal disease foreign to the United States within 48 h after discovery. Although the ostensible focus of this plan is to protect the health of the national livestock herd, the proposed tracking system has the potential to benefit public health through improved traceability of all food animal products. The plan requires an information system and infrastructure that allow for the identification of individual or groups of animals and of premises, and it requires a record of the animal's termination to be retained by the processing plant (slaughterhouse). The system should include a requirement that trace-back information be made readily available to both animal health and public health regulatory officials during foodborne disease investigations.

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