A Multistate Outbreak of *E. coli* O157:H7 Infection

STUDENT'S VERSION

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NOTE: This case study is based on two real-life outbreak investigations undertaken in Michigan and Virginia in 1997. Some aspects of the original outbreaks and investigations have been altered, however, to assist in meeting the desired teaching objectives and allow completion of the case study in less than 3 hours.

Students should be aware that this case study describes and promotes one particular approach to foodborne disease outbreak investigation. Procedures and policies in outbreak investigations, however, can vary from country to country, state to state, and outbreak to outbreak.

It is anticipated that the epidemiologist investigating a foodborne disease outbreak will work within the framework of an "investigation team" which includes persons with expertise in epidemiology, microbiology, sanitation, food science, and environmental health. It is through the collaborative efforts of this team, with each member playing a critical role, that outbreak investigations are successfully completed.

Please send us your comments on this case study by visiting our website at http://www.phppo.cdc.gov/phtn/casestudies. Please include the name of the case study with your comments.

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Public Health Service
Centers for Disease Control and Prevention
Atlanta, Georgia 30333

STUDENT'S VERSION
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Learning objectives:

After completing this case study, the student should be able to:

1. describe the unique role the laboratory can play in the detection and investigation of a foodborne disease outbreak
2. perform in-depth interviews of selected case-patients to generate hypotheses about the source of an outbreak and mode of transmission
3. determine the most efficient epidemiologic study design to test a hypothesis (including the case definition and appropriate comparison group)
4. list three ways to select a comparison group for a study and the advantages and disadvantages of each method
5. list detailed product information that will facilitate a traceback procedure
6. discuss the relative merits of an intervention based on changes in product processing (or design) versus changes in consumer or producer behaviors

PART I - OUTBREAK DETECTION

Escherichia coli O157:H7 was first identified as a human pathogen in 1982 in the United States of America, following an outbreak of bloody diarrhea associated with contaminated hamburger meat. Sporadic infections and outbreaks have since been reported from many parts of the world, including North America, Western Europe, Australia, Asia, and Africa. Although other animals are capable of carrying and transmitting the infection, cattle are the primary reservoir for *E. coli* O157:H7. Implicated foods are typically those derived from cattle (e.g., beef, hamburger, raw milk); however, the infection has also been transmitted through contact with infected persons, contaminated water, and other contaminated food products.

Infection with *E. coli* O157:H7 is diagnosed by detecting the bacterium in the stool. Most laboratories that culture stool do not routinely test for *E. coli* O157:H7, but require a special request from the health care provider. Only recently has *E. coli* O157:H7 infection become nationally notifiable in the U.S. Outside the U.S., reporting is limited to a few but increasing number of countries.

In the last week of June 1997, the Michigan Department of Community Health (MDCH) noticed an increase in laboratory reports of *E. coli* O157:H7 infection. Fifty-two infections had been reported that month, compared with 18 in June of 1996. In preliminary investigations, no obvious epidemiologic linkages between the patients were found. The increase in cases continued into July.

Question 1A: What could account for the increase in cases reported to MDCH?

Question 1B: What information might help determine which of these explanations is the most likely cause of the increased numbers?

Laboratory subtyping can help determine if an increased number of isolates of the same bacterial species results from a common source outbreak. Subtyping methods are based on selected biologic and/or genetic characteristics of bacteria that tend to differ between isolates of the same species. In a common source outbreak, however, isolates typically arise from the same parent organism. These isolates will be similar to each other with respect to these biologic and genetic characteristics and have similar subtyping results.

One subtyping method is DNA "fingerprinting" by Pulsed Field Gel Electrophoresis (PFGE). In DNA fingerprinting, the bacterial DNA is cut into pieces. The pieces are separated by placing them in a jelly-like substance (i.e., the gel), acting as a sieve, to which a pulsing electric field is applied. The electric field drives the DNA pieces across the gel over a period of hours. The smaller pieces move through the gel more quickly and the larger pieces more slowly resulting in a separation of the DNA into distinct bands. The bands are made to fluoresce and are read under ultraviolet illumination. This DNA "fingerprint" resembles a bar code. (Figure 1)

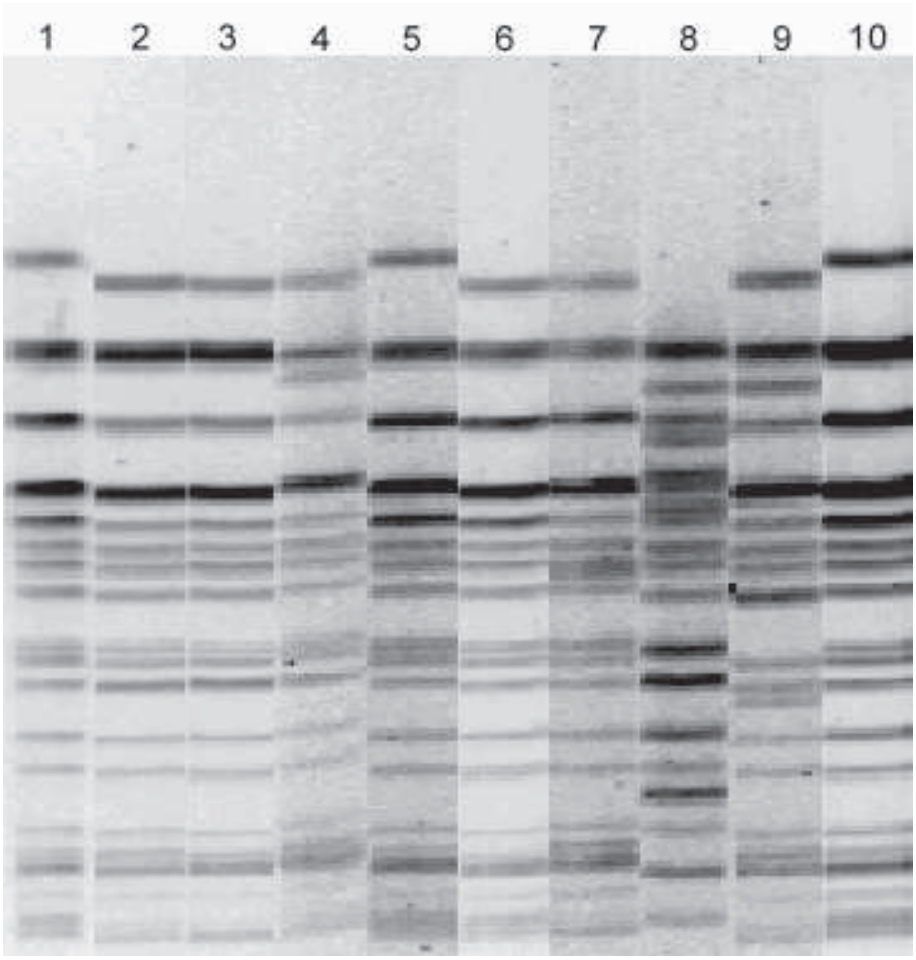
Figure 1. Typical DNA banding pattern resulting from PFGE.

Different DNA composition will result in different PFGE banding patterns. Bacteria descended from the same original parent will have virtually identical DNA and their DNA fingerprints will be indistinguishable. Identification of a cluster of isolates with the same PFGE pattern suggests that they arose from the same parent and could be from the same source.

Similar DNA fingerprints alone, however, are insufficient to establish a linkage between isolates and a common source outbreak. An epidemiologic investigation is necessary to demonstrate that there is a common source and to identify it. To be most useful, PFGE subtyping needs to be performed on a routine basis, in real time, so that results are available (and reviewed) soon after a case is first detected.

Question 2: Compare the DNA fingerprints in Figure 2 from seven of the Michigan *E. coli* O157:H7 cases. Each isolate has its own vertical lane (i.e., column). Controls appear in lanes #1, 5, and 10. Which Michigan isolates appear similar?

Figure 2. PFGE results on *E. coli* O157:H7 isolates from Michigan, June-July 1997.



DNA fingerprinting, performed in the MDCH State Laboratory during the second week of July showed that 17 of the first 19 *E. coli* O157:H7 isolates from June-July were indistinguishable. They did not match any fingerprints from a convenience sample of isolates from patients with *E. coli* O157:H7 infection before May.

Based on the PFGE findings, MDCH suspected the cases of *E. coli* O157:H7 infection resulted from a common source. On July 15, MDCH initiated an investigation. The Centers for Disease Control and Prevention (CDC) was asked to join the investigation.

PART II - DESCRIPTIVE EPIDEMIOLOGY AND HYPOTHESIS GENERATION

The incubation period for *E. coli* O157:H7 ranges from 3-8 days with a median of 3-4 days. The infection often causes severe bloody diarrhea and abdominal cramps, but can also cause a nonbloody diarrhea or result in no symptoms. In some persons, particularly children under 5 years of age and the elderly, the infection can cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2-7% of infections lead to this complication.

For the outbreak investigation in Michigan, a case was defined as diarrhea (≥3 loose bowel movements a day) and/or abdominal cramps in a resident of Michigan with onset of symptoms between June 15 and July 15 and a stool culture yielding *E. coli* O157:H7 with the outbreak strain PFGE pattern.

Question 3: What are the advantages and disadvantages of this case definition? How might you change it?

Of the initial 38 persons who met the case definition, 26 (68%) were female with a median age of 31 years. (Table 1)

Table 1. Age group and gender distribution for persons with *E. coli* O157:H7 infection and the outbreak PFGE pattern, Michigan, June 15 - July 15, 1997. (N=38)

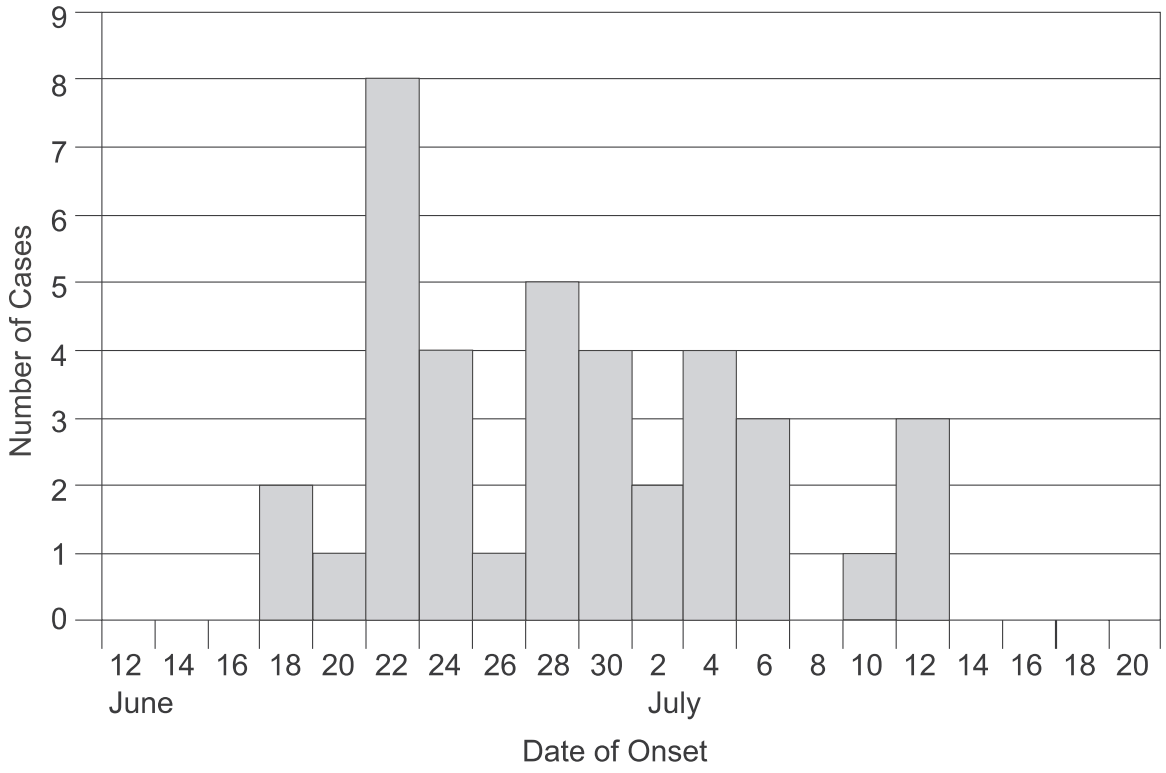
Age group (years)	Gender		TOTAL
	Male	Female	
0-9	2 (17%)*	2 (8%)	4 (11%)
10-19	2 (17%)	3 (12%)	5 (13%)
20-39	3 (25%)	9 (35%)	12 (32%)
40-59	2 (17%)	8 (31%)	10 (26%)
60+	3 (25%)	4 (15%)	7 (18%)
TOTAL	12 (101%)	26 (101%)	38 (100%)

* percentages refer to column totals.

Question 4: Compare the age and gender distribution of *E. coli* O157:H7 cases from the Michigan outbreak and those reported from U.S. FoodNet sites in 1997. (see Appendix 1)

The 38 cases of *E. coli* O157:H7 infection meeting the investigation case definition were reported from 10 counties in the lower peninsula of Michigan. Onset of illness occurred from mid-June to mid-July, peaking on June 22. (Figure 3)

Figure 3. Date of illness onset for persons with *E. coli* O157:H7 infection and the outbreak PFGE pattern, Michigan, June 15 - July 15, 1997. (N=38)



From July 16 - 19, hypothesis-generating interviews were undertaken with seven patients. These patients lived in four different counties and ranged in age from 5-69 years. Three of the patients were female.

Question 5: What kinds of questions would you ask in the hypothesis-generating interviews? Be sure to consider all possible modes of transmission of *E. coli* O157:H7.

Question 6: Working in groups of 2-4 students, role play a hypothesis-generating interview of one of the case-patients. One student should play the patient and will be given information about that patient (see Appendix 2 "Patient #1" and "Patient #2"). Another student should play the investigator and will interview the patient. Efforts should be made to simulate a real interview based on the information provided. After 15 minutes, you will be asked to share your experience in interviewing the patient. (If time permits, students can switch roles and a second "patient" can be interviewed using material in Appendix 2.)

Hypothesis-generating interviews revealed that most cases had consumed lettuce and alfalfa sprouts in the week before they became ill. No single restaurant or social event was identified in common.

Question 7: Given your knowledge about *E. coli* O157:H7, the descriptive epidemiology of the initial cases, and the results of hypothesis-generating interviews, outline the information available at this point on the source of the outbreak and mode of transmission and state your leading hypothesis.

PART III - DESIGNING AN EPIDEMIOLOGIC STUDY TO TEST THE HYPOTHESIS

To test the hypothesis on the source of the outbreak, MDCH and CDC conducted a case-control study from July 21-27. Thirty-one of the initial 38 persons meeting the original case definition (i.e., those not used in hypothesis generating interviews) were included as cases. It was decided that two controls would be selected for every case and would be matched to the case by age group (0-<2 years, 2-<5 years, 5-<12 years, 12-<18 years, 18-<60 years, and 60+ years) and gender.

Question 8A: How would you define controls for this study?

Question 8B: Do you agree with the investigators' decision to match on age group and gender? Why or why not?

Question 9: What methods might be used to identify controls? What are the advantages and disadvantages of each method?

Question 10: Over what time period would you examine exposures to possible risk factors for cases? For controls?

The investigators identified controls for the study using sequential digit dialing. Exposure information among cases was collected for the 7 days before onset of illness. For controls, exposure information was collected for the 7 days before the interview and for the 7 days before the onset of illness in the matching case.

Twenty-seven case-control sets were interviewed; the remaining case-patients could not be reached.

PART IV - ANALYSIS AND INTERPRETATION OF EPIDEMIOLOGIC RESULTS

In the case-control study, 15 (56%) of 27 ill persons reported eating alfalfa sprouts in the 7 days before onset of illness, but only three (6%) of 53 controls reported eating them in the 7 days before the interview (matched odds ratio [MOR]: 27, 95% confidence interval 5-558.) When controls were asked about alfalfa sprout consumption for the same 7-day interval as ill persons, a similar association was observed; four of 53 controls reported eating sprouts (MOR 25, 95% CI 4-528.) No other food item was significantly associated with illness.

Question 11: What are possible explanations for the association between illness and sprouts?

Question 12: How might you explain the 12 ill persons in the study who did not report eating alfalfa sprouts in the 7 days before they became ill?

Question 13: What control measures might you consider at this point? What further studies might you suggest? (See Appendix 3 for a description of alfalfa sprouts and the typical sprouting procedure.)

PART V - OTHER INVESTIGATIONS

Tracebacks of food are often necessary to identify sources of contamination and quickly limit a public health threat by removing these sources. One purpose of a traceback is to ascertain the distribution and production chain for a food product so that an effective recall can be undertaken. Tracebacks can also clarify the point or points at which the implicated food was likely to have become contaminated and help determine how to prevent similar outbreaks in the future. Epidemiologic tracebacks can accomplish each of these goals, but are different from the more detailed, regulatory tracebacks which follow rules of legal evidence.

An epidemiologic traceback usually begins with the information available at the time of purchase of the implicated food item and extends back to the very beginning of its production. All production steps, from harvest to consumption, are examined.

Full tracebacks leading to formal product recalls can be time-consuming and result in many dead-ends. Pertinent information and records are often missing or poorly maintained. Traceback efforts may require hundreds of hours of tedious work and may extend to other states and countries.

Question 14: What criteria should be considered before deciding to undertake a traceback procedure? Would you consider doing a traceback in the Michigan outbreak?

MDCH and CDC decided to do an epidemiologic traceback of the alfalfa sprouts implicated in the Michigan outbreak.

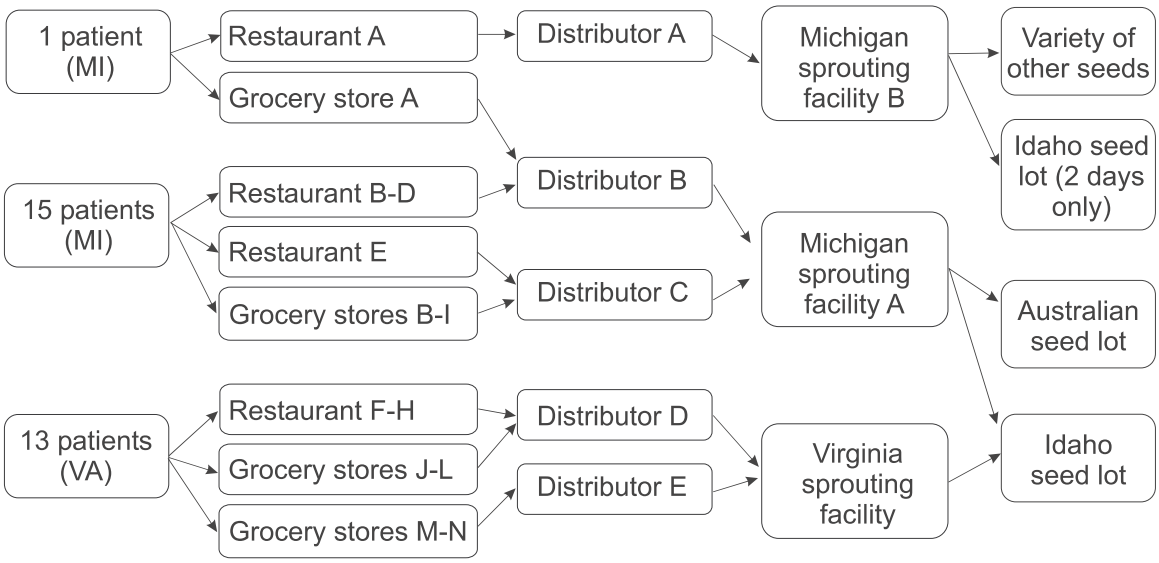
Question 15: What information on the implicated food item might facilitate the traceback process?

Of the 16 patients who ate sprouts for whom the source of the sprouts could be traced, 15 led to a single sprouting facility, facility A in Michigan; in the remaining traceback, the patient could have eaten sprouts from either facility A or facility B in Michigan. (Figure 4) Facility A and B were the only facilities that sprouted alfalfa seed in the state. Sprouts grown by facility A at the time of the outbreak came from two lots of seed: one from Idaho and one from Australia.

At this point, the investigators became aware of a concurrent outbreak of *E. coli* O157:H7 infection in Virginia. CDC subtyped the strains from Virginia and identified the same PFGE pattern as in the Michigan outbreak. A case-control study conducted by the Virginia Department of Health (VDH) linked the concurrent outbreak of O157:H7 infections to alfalfa sprouts.

In Virginia, the source of sprouts could be traced for 13 patients; all led to one sprouting company in Virginia. (Figure 4) The Virginia sprouting company was using a single lot of seed harvested in Idaho -- the same lot as the one used at facility A in Michigan. Traceback of the seed to the distributor identified it as part of a 17,000 pound lot of which 6,000 pounds still remained.

Figure 4. Traceback results of the *E. coli* O157:H7 investigation of alfalfa sprouts in Michigan (MI) and Virginia (VA), 1997.



Question 16: Given the results of the Michigan and Virginia traceback investigations, where is the most likely point of contamination in the production of the sprouts?

The implicated seed lot was a blend of 5 lots from fields of four farmers and was harvested between 1984 and 1996. The seed processor and the farmers were located in Idaho.

Question 17: In inspecting the alfalfa fields and harvesting process, what possible points of contamination should you consider?

Inspection of the alfalfa fields revealed three possible sources of contamination: cattle manure, irrigation water, and deer feces. Although manure is not normally applied to alfalfa fields in Idaho, cattle feed lots were common in this area and the alfalfa fields of one farmer were adjacent to a feed lot. Manure may have leaked or been illegally dumped onto the alfalfa fields or run-off water from neighboring fields, contaminated by manure, may have been used to irrigate the alfalfa fields. In addition, three of four farmers occasionally saw deer in their fields and one field was situated next to a wildlife refuge.

The seed from each of the farmers was harvested and mechanically cleaned at the same seed processing plant. The seeds were then placed in 50 lb. bags. No further processing occurred. Most of the seed was produced to plant alfalfa fields (e.g., to produce hay for livestock feed); the relatively small amount of seed used for sprouting was not handled any differently than the raw agricultural commodity seed.

Question 18: What interventions/control measures would you suggest at this point?

PART VI - CONTROL

The implicated seed lot was not distributed to any other sprouting companies in or outside the United States. The remaining 6,000 lbs. of seed was immediately removed from the marketplace. A sample of 500 grams of seed was cultured directly, and the same amount was sprouted and then cultured; neither yielded *E. coli* O157:H7.

The Idaho Division of Food and Drugs held meetings at which public health officials explained to seed growers the need to protect alfalfa and other seeds used in sprouting from contamination during growing, harvesting, and packing. Both MDCH and the VDH made public television and radio announcements about the risk of contaminated sprouting seeds and recommended that persons at high risk for complications from *E. coli* O157:H7 infection not eat sprouts.

The Center for Food Safety and Quality Enhancement began working with the sprout industry to identify ways to make sprouts safer for human consumption. In tests with artificially inoculated seed, treating the seed by soaking it in a chlorine solution* (2000 ppm hypochlorite in 57-60°C water) at the time of sprouting reduced the level of contamination by a thousand-fold. Irradiation has also been tested and appears to work well in decontaminating sprout seeds. However, this treatment leads to diminished sprouting ability and has not been approved by the FDA.

Question 19: What type of intervention is likely to be most effective against the problem of sprout contamination: education of producers, education of consumers, or changes in methods of product processing? Why?

*Chemical treatment with a hypochlorite solution is a U.S. Food and Drug Administration (FDA) approved treatment of foods.

EPILOGUE

In Michigan, demographic characteristics differed among cases reporting consumption of alfalfa sprouts and those who did not. The median age of non-sprout eaters was 12 years compared with 38 years for sprout eaters. Onset of illness among non-sprout eaters occurred between June 30 and July 13, with most sprout-related cases occurring in June.

On interview, it was revealed that seven of the non-sprout eating cases, all children, had swum in the same man-made lake during the July Fourth holiday weekend or the weekend before. Because *E. coli* O157:H7 can survive for weeks in lake water and has a very low infectious dose, the outbreak investigators hypothesized that the lake was contaminated by feces from a patient with illness from sprouts. Children could have acquired illness by swallowing water while swimming or some other exposure that occurred among persons swimming at the lake (e.g., concessions, person-to-person). Testing of the lake water on June 24 and July 7 did not reveal elevated levels of *E. coli*.

This outbreak illustrates several important concepts in the investigation of foodborne diseases:

1) The finding of a second mode of transmission among patients with the same DNA fingerprint emphasizes that new subtyping methods such as PFGE are tools to improve investigations but cannot substitute for a thorough epidemiologic workup.
2) Secondary spread of the outbreak strain of *E. coli* O157:H7 through recreational waters (or some associated activity) illustrates how a foodborne disease outbreak can extend into the community and affect those who do not consume the contaminated food.
3) The discovery of a new vehicle for the transmission of *E. coli* O157:H7 demonstrates how changes in the food industry have made the control of foodborne diseases more complex and challenging. New food products are not always accompanied by practices to ensure their safety.
4) The multistate nature of this outbreak, indicative of the wide distribution of food products in today's market, shows how foodborne disease outbreaks can affect persons simultaneously in widely separated locations. This means not only foodhandling practices but disease and outbreak investigation efforts in one part of the world can readily affect persons in another part.
5) And, finally, the lengthy genesis and conclusion to this outbreak (i.e., cases were first recognized in June, 1997 and continued to occur as late as September, 1997) suggest the need for improved investigation of foodborne diseases. Among other things, more reliable case reporting, routine performance of PFGE on *E. coli* O157:H7 isolates, and the examination and comparison of results in real-time will increase the rate of response to foodborne diseases and decrease the number of people affected by them.

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APPENDIX 1. Distribution of *E. coli* O 157:H7 cases reported to FoodNet Sites* by age group and gender, United States, 1997. (N=340)

Age group (years)	Gender		TOTAL
	Male	Female	
0-<1	5 (3%)	5 (3%)	10 (3%)
1-9	77 (48%)	77 (43%)	154 (45%)
10-19	36 (22%)	18 (10%)	54 (16%)
20-29	10 (6%)	20 (11%)	30 (9%)
30-39	6 (4%)	12 (7%)	18 (5%)
40-49	7 (4%)	5 (3%)	12 (4%)
50-59	7 (4%)	17 (10%)	24 (7%)
60+	14 (9%)	24 (13%)	38 (11%)
TOTAL	162 (100%)	178 (100%)	340 (100%)

*Foodborne Diseases Active Surveillance Network (FoodNet) is a collaborative project between CDC, the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA), and selected state and local health departments. In 1997, FoodNet conducted population-based active surveillance for confirmed cases of *Campylobacter*, *Escherichia coli* O157, *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia* infections in Minnesota, Oregon, and selected counties in California, Connecticut, and Georgia (total population: 16.1 million).

APPENDIX 2. Background information for role-playing interviews of patients (see Question 6)

PATIENT #1: Helen Dresher

Age: 25 years Sex: Female
Address: City: 35 County: 12
Phone number: (248) 555-0991 (home) (248) 555-1423 (work)

works as office manager for local legal firm, lives in apartment by herself

ILLNESS
Onset of symptoms: 6/22/97
Symptoms: bloody diarrhea, abdominal cramps, vomiting, (no fever)
Duration of symptoms: 5 days
Initially treated with Pepto-Bismal and Imodium AD
Visited Dr. Locke on 6/25/97
Treatment received: ciprofloxacin

PAST MEDICAL HISTORY
No antibiotics in two weeks before illness
No antacids in two weeks before illness
No chronic illness

EXPOSURES IN 7 DAYS BEFORE ILLNESS:
No travel outside Michigan
No swimming or wading in recreation areas
Did not drink water from a private well
No contact with animals

FOOD HISTORY FOR 7 DAYS BEFORE BECOMING ILL:
High risk foods:
Hamburgers: NEG Ground beef: NEG Raw or unpasteurized milk: NEG

6/15 (Sunday)
Breakfast: coffee with cream
Lunch: yogurt, diet coke
Dinner: baked chicken, potatoes, salad with lettuce, cucumbers, carrots, green peppers

6/16 (Monday)
Breakfast: coffee with cream
Lunch: yogurt, diet coke
Dinner: oriental vegetables with beef, rice, iced tea

6/17 (Tuesday)
Breakfast: coffee with cream
Lunch: left-over baked chicken, pita bread, sprouts (alfalfa), cucumbers, diet coke
Dinner: noodles in cream sauce, green beans, iced tea

6/18 (Wednesday)
Breakfast: English muffin, coffee with cream
Lunch: yogurt, diet coke
Dinner: salad (lettuce, cherry tomatoes, celery, cheese chunks), crackers, diet ranch salad dressing

6/19 (Thursday)
Breakfast: coffee with cream
Lunch: bean burrito, diet coke
Dinner: pasta with shrimp and snow peas, iced tea

6/20 (Friday)
Breakfast: coffee with cream
Lunch: chicken salad sandwich, tomato, sprouts (alfalfa), pickle spear, diet coke (5th Street Diner)
Dinner: broiled fish, rice, salad (lettuce, spinach, carrots), vinaigrette dressing
Party: variety of cheese and crackers, white wine

6/21 (Saturday)
Breakfast: coffee with cream
Lunch: bagel, cream cheese, potato chips, oreos, ding dongs, dove bar, potato chips, M&Ms, diet coke
Dinner: skip

NAMES AND LOCATIONS OF RESTAURANTS OR CAFETERIA'S WHERE ATE IN THE 7 DAYS BEFORE ILLNESS:
5th Street Diner City: 35

USUAL STORES OR MARKETS: (does own shopping)
Store: 1 (City: 35) Store: 2 (City: 35) Store: 3 (City: 35)

STORES OR MARKETS FOR PRODUCE:
Store: 1 (City: 35) Store: 2 (City: 35)

OTHER SPECIAL EVENTS: party at friends on 6/20

OTHER ILL PERSONS: no family members or acquaintances ill

PATIENT #2: G. Warren Wilson

Age: 69 years Sex: Male
Address: City: 5 County: 5
Phone number: (616) 555-1547 (home)

lives with wife and elderly mother-in-law, retired tire salesman, wife prepares all the food

ILLNESS
Onset of symptoms: 6/25/97
Symptoms: bloody diarrhea, abdominal cramps, (no vomiting or fever)
Duration of symptoms: 7 days
No antidiarrheal medications
Visited Dr. Smith on 6/29/97 (concerned about perforated diverticulum), hospitalized for 4 days
Treatment received: antibiotics, intravenous fluids, transfusion

PAST MEDICAL HISTORY
No antibiotics in two weeks before illness
No antacids in two weeks before illness
Diverticulitis, gastric ulcer disease, smokes

EXPOSURES IN 7 DAYS BEFORE ILLNESS:
No travel outside Michigan
No swimming or wading in recreation areas
Drinks water from private well (source of water for home)
No contact with animals (wife has 7 cats which do not come into house)

FOOD HISTORY FOR 7 DAYS BEFORE BECOMING ILL:
High risk foods:
Hamburgers: NEG Ground beef: NEG Raw or unpasteurized milk: NEG

6/18 (Wednesday)
Breakfast: fried eggs, bacon, toast, coffee (Sanka), preserves
Lunch: vegetable soup, bread, oatmeal cookies, coffee (Sanka)
Dinner: baked ham, scalloped potatoes, green beans, spinach salad, rolls, butter, ice cream

6/19 (Thursday)
Breakfast: fried eggs, bacon, toast, coffee (Sanka), preserves
Lunch: ham sandwich with Swiss cheese and mustard, corn chips, oatmeal cookies, coffee (Sanka)
Dinner: fried chicken, mashed potatoes, lettuce and tomato salad, corn-on-the cob, canned peaches, rolls, butter, ice cream

6/20 (Friday)
Breakfast: fried eggs, bacon, toast, coffee (Sanka), preserves
Lunch: tuna salad sandwich with mayonnaise, sprouts (alfalfa), tomato, pickle, potato chips, oatmeal cookies, coffee (Sanka)
Dinner: broiled steak, French fries, salad (lettuce, tomato, carrots, celery, red cabbage, mushrooms), thousand islands salad dressing, bread, butter, ice cream

6/21 (Saturday)
Breakfast: fried eggs, bacon, toast, hash brown potatoes, coffee (Sanka), preserves
Lunch: ham sandwich, coleslaw, chocolate chip cookies, coffee (Sanka)
Dinner: pan fried pork chops, mashed potatoes, green beans, rolls, butter, ice cream

6/22 (Sunday)
Breakfast: waffles, syrup, sausage, coffee (Sanka)
Dinner: pot roast with roasted potatoes and vegetables, apple sauce, bread, butter, peach pie, ice cream

6/23 (Monday)
Breakfast: fried eggs, bacon, toast, coffee (Sanka), preserves
Lunch: roast beef sandwich with mayonnaise and sprouts (alfalfa), corn chips, peanut butter cookies, coffee (Sanka)
Dinner: round steak, parsley potatoes, zucchini, bread, butter, ice cream

6/24 (Tuesday)
Breakfast: fried eggs, bacon, toast, coffee (Sanka), preserves
Lunch: roast beef sandwich with Swiss cheese and horse radish, coleslaw, chocolate chip cookies, coffee (Sanka)
Dinner: chicken and rice casserole, green beans, fruit cocktail, bread, butter, ice cream

NAMES AND LOCATIONS OF RESTAURANTS OR CAFETERIA'S WHERE ATE IN THE 7 DAYS BEFORE ILLNESS: did not eat out

USUAL STORES OR MARKETS: (wife does all of the shopping)
Store: 5 (City: 49) Store: 24 (City: 49) Store: 2 (City: 49)

STORES OR MARKETS FOR PRODUCE:
Store: 5 (City: 49)

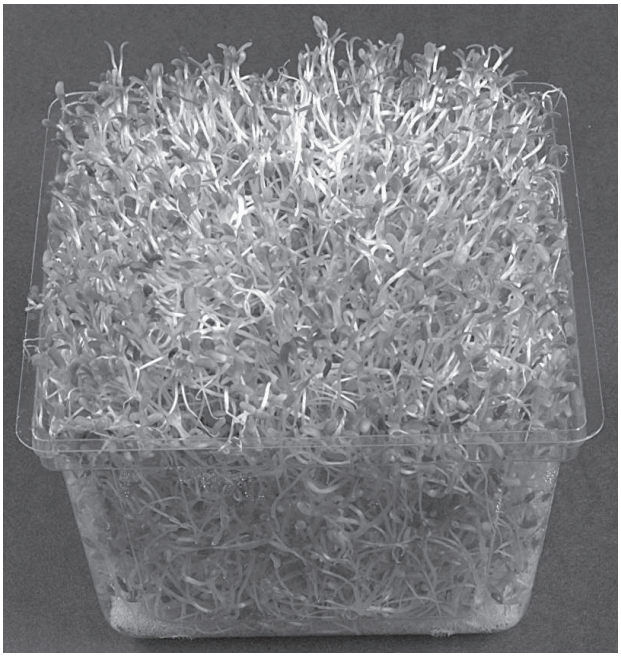
OTHER SPECIAL EVENTS: square dancing at senior citizens center (no food served)

OTHER ILL PERSONS: wife and mother-in-law not feeling well, loose stools and abdominal cramps

APPENDIX 3. Alfalfa sprouts

Alfalfa sprouts are produced for human consumption through the germination of alfalfa seeds in a moist, non-soil environment. Like sprouts from many other seeds, alfalfa sprouts are not cooked and are consumed within a few days of sprouting. Alfalfa sprouts are more delicate than other seed sprouts and are used in salads and as a garnish, often to add texture and moisture.

Photograph 1. Alfalfa sprouts 5 days after germination.



The following method (or a facsimile) is used to sprout alfalfa seeds both commercially and by private individuals:

1) The seeds are rinsed in water. (Many producers use a solution of water and household chlorine.)
2) The seeds are covered with water and allowed to soak over night (for about 12 hours).
3) The water is drained and the seeds are placed in sprouting trays (or a jar) where they continue to drain.
4) The seeds are rinsed (or misted) with water twice daily until they sprout and reach the desired length (approximately 2-5 days).
5) After reaching the desired length, the sprouts are removed and rinsed.
6) Excess moisture is removed.
7) The sprouts are placed in a container, covered, and stored in the refrigerator.