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

INSTRUCTOR'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
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Target audience: epidemiologists and other persons with knowledge of basic epidemiologic concepts and experience in data collection and analysis who are interested in learning specific skills for investigating infectious disease outbreaks

Training prerequisites: descriptive epidemiology, epidemic curves, measures of association, study design, outbreak investigation. The student will also benefit from having some familiarity with food microbiology and environmental investigation techniques but will be likely to rely heavily on others with greater expertise in these areas in a real-life outbreak situation.

Teaching materials required: calculator

Time required: approximately 2 hours and 30 minutes

Language: English

Level of case study: Basic ___ Intermediate _X_ Advanced ___

Materials borrowed from:
"Foodborne Illness Investigation and Control Reference Manual", Massachusetts Department of Public Health, Division of Epidemiology and Immunization, Division of Food and Drugs, and Division of Diagnostic Laboratories (1997)

"Guidelines for the Investigation and Control of Foodborne Disease Outbreaks", World Health Organisation, Food Safety Unit Division of Food and Nutrition and Division of Emerging and Other Communicable Diseases Surveillance and Control (DRAFT, 1999)

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INSTRUCTOR'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?

It may be useful to categorize reasons for the increase as those causing an "artificial (or perceived) increase" in number of infections vs. those causing a "real increase".

Artificial increase:
- *increased culturing of stools*
- *initiation of new testing by the laboratory (i.e., lab did not undertake necessary procedures to isolate this organism in the past)*
- *laboratory error in identification*
- *contamination of cultures*
- *changes in reporting procedures*
- *errors in data entry*

Real increase:
- *an increase in population size*
- *changes in population characteristics (with an influx of persons at higher risk for the infection)*
- *an increase in rate of infection due to random variation (fluctuation) in incidence (i.e., chance)*
- *an increase in rate of infection due to an outbreak (NOTE: This latter situation could result from a common source exposure or an increase in behaviors [e.g., outdoor cooking] that lead to increased infections from a variety of sources.)*

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

If not already known, it would be helpful to consult with staff from the laboratory and surveillance section (and other key informants) to collect the following information:
- *changes in local laboratory procedures or staff*
- *if problems with stool culturing have been identified*
- *changes in physician diagnostic practices*
- *changes in laboratory or physician reporting practices (e.g., changes in mandatory reporting requirements, recent efforts to increase reporting through provider education)*
- *changes in population demographics*
- *characteristics of cases (e.g., clustering in space, time, or person)*
- *subtyping of the isolates to see if they are the same/related*

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)

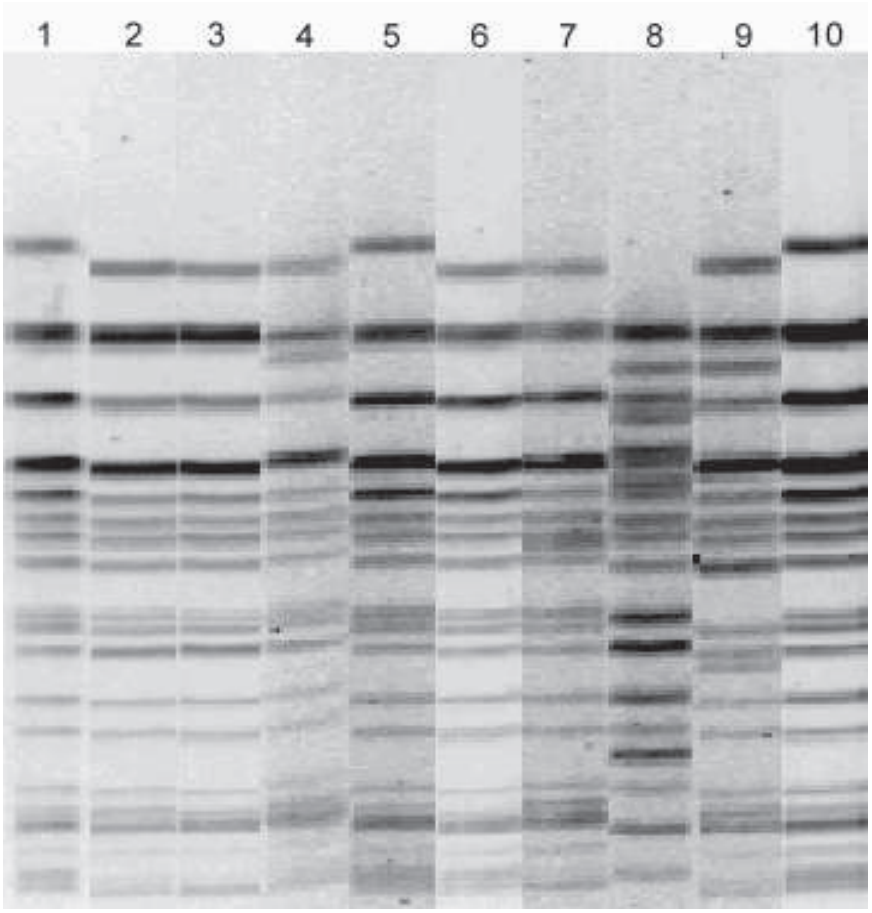
Figure 1. Typical DNA banding pattern resulting from PFGE.

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.



Typically, a PFGE "pattern" is defined as having the same banding pattern but including up to one band difference. By this definition, isolates #2, 3, 4, 6, and 7 are indistinguishable by these PFGE results. (Isolate #4 differs by one band.)

NOTE: To facilitate routine examination of PFGE results, the U.S. Centers for Disease Control and Prevention (CDC) is currently equipping State Public Health Laboratories with the capacity to perform and compare PFGE results on selected foodborne pathogens. Laboratories participating in the network, called PulseNet, perform PFGE on disease-causing bacteria isolated from humans and suspected food using standardized equipment and methods. Once

PFGE patterns are generated, they are entered into an electronic database of DNA "fingerprints" at the state or local health department and transmitted to CDC where they are filed in a central computer. The system will ultimately be developed into a national online database. If patterns submitted by laboratories in different locations during a defined time period are found to match, the CDC computer will alert PulseNet participants of a possible multistate outbreak so that a timely investigation can be done.

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?

A case definition is a standard set of criteria for deciding whether an individual should be classified as having the disease of interest. A case definition includes clinical criteria (e.g., signs, symptoms, and laboratory tests) and restrictions on time, place, and person.

For the case definition used in the Michigan investigation:
Advantages:

- *Lab confirmation will increase the specificity of the case definition (and exclude cases that might not be related to the outbreak). This reduces misclassification and maximizes the power to detect a source of the outbreak.*

Disadvantages:

- *Lab confirmation will exclude patients who did not see a doctor, patients who were not cultured, and cultured patients without PFGE testing. Lab confirmation will decrease the sensitivity of the case definition and, possibly, lead to a misrepresentation of case characteristics.*
- *Limiting cases to Michigan residents may be practical from the standpoint of a state-based investigation but may exclude visitors who became infected or inhibit investigators from recognizing an extension of the outbreak into other states.*

We are not given enough information to say whether the dates are reasonable. A line listing of cases might be helpful. Confining the dates of onset to June 15-July 15 could limit the number of secondary cases (e.g., person-to-person transmission) included in the study that could interfere with identification of the initial source of the outbreak.

The general purpose of including symptoms (as well as laboratory confirmation) in the case definition for gastrointestinal illnesses is to exclude persons who are chronic carriers of the infection (such as for salmonellosis). It is unlikely to impact this study, however, since most persons with E. coli O157:H7 will have symptoms and prolonged carriage of E. coli is uncommon.

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)

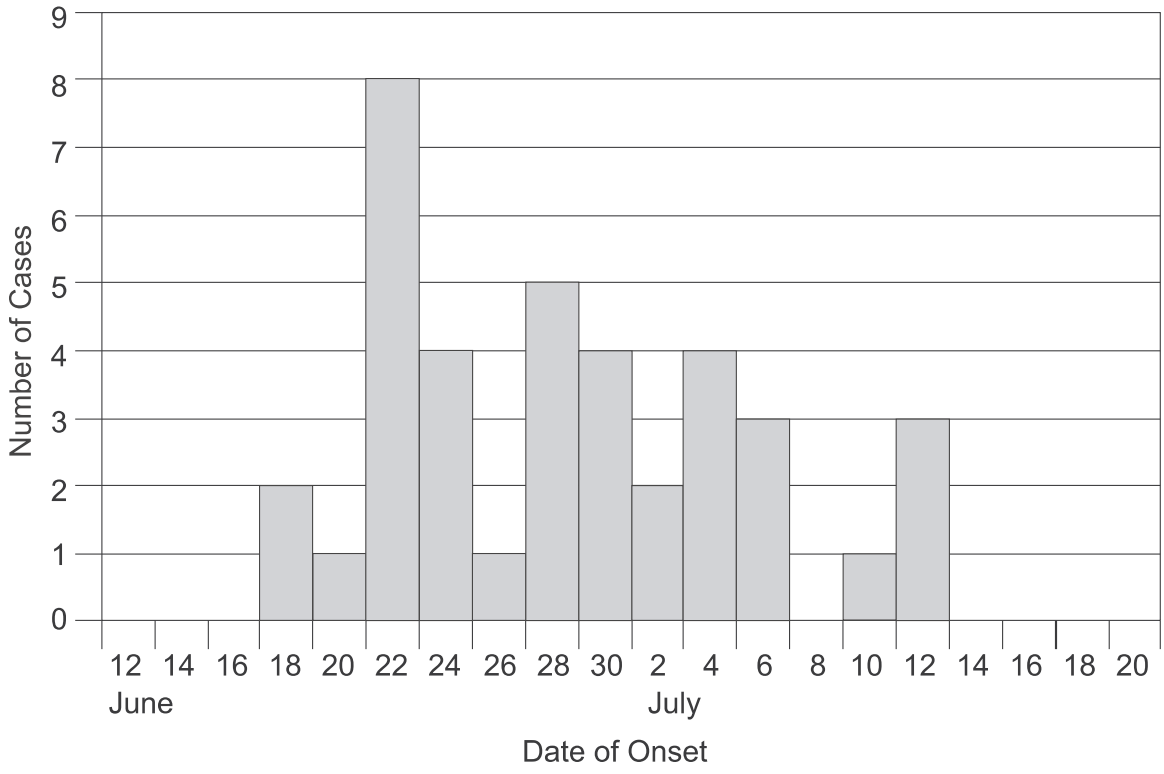
It may help to have students graph the two age-gender distributions to facilitate their comparison.

Among cases reported to FoodNet in 1997, the male:female ratio was similar (1:1.1). Rates of infection were highest among children and declined with increasing age (until about 50 years of age where rates again increased). In contrast, the cases in Michigan (with the outbreak PFGE pattern) were more common among adult females, 20-59 years of age. This age and gender

distribution may suggest an unusual source of infection such as a product used more commonly by young or middle-aged women. It might also be consistent with infection among mothers exposed to diapered children, although one would expect to see more cases among children in that situation.

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.

The objective of hypothesis-generating interviews is to explore all potential sources of infection with a limited number of patients. Commonalities among these patients (as well other information collected early in the outbreak) will allow the development of hypotheses about the source of the outbreak. These hypotheses can then be specifically tested in subsequent epidemiologic (and other) studies.

Exposure to both common and less common sources of infection should be examined. Because the typical incubation period for E. coli O157:H7 is 3-4 days, but ranges from 3-8 days, exposures in the 7 days before onset of illness should be considered. Primary areas of focus include:

- *demographic information*
- *clinical details of the illness with date of onset, duration, and severity of symptoms*
- *visits to health care providers or hospitals, and laboratory results*
- *a complete food history in the last 7 days*
- *water exposure in the last 7 days (e.g., drinking water, exposure to recreational waters)*
- *exposure to other ill persons in the last 7 days*
- *exposure to children in day care in the last 7 days*
- *exposure to a farm or farm animals in the last 7 days*
- *travel outside the immediate area in the last 7 days*

In taking the food history, patients should be asked about foods eaten in their home as well as at restaurants, fast food establishments, delis, and the homes of friends and family. The names and addresses of commercial food serving establishments should be collected. The date and time of food consumption and any unusual observations should be noted.

The interviewer should ask the patient to use a calendar or appointment book in providing the food history; focusing on prominent events, weekends, or holidays may help jog the patient's memory. One efficient way to review the food history is to try to reconstruct each day in the time period of interest, meal by meal.

In addition to the more open ended questions about exposures, patients should be asked specifically about consumption of food items and exposures that have been linked to E. coli O157:H7 infection in previous studies (e.g., hamburgers, ground beef, milk, water).

For hypothesis-generating interviews to be most informative, an effort should be made to interview a variety of patients (i.e., with different demographic characteristics).

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.)

To allow the students to talk more privately and not interfere with the other students, additional rooms or sites may need to be identified in which to do the mock interviews. Students may need a little help in getting started. The instructor should encourage the student who is playing the patient to "get into the part" and ad lib as required. They should not divulge the information provided for their character, however, unless asked by the interviewer.

When the class reassembles, the instructor should ask the students how the "interviews" went and discuss techniques for good interviews. Issues to address include the need to:

1) *establish the interviewer's credentials,*
2) *develop a rapport with the patient*
3) *organize one's approach and systematically collect the desired information (without jumping around from subject to subject)*
4) *conclude the interview (remember to thank the patient!) and provide the patient with a means to contact the interviewer in the future*

Hopefully, the students will note that the information is difficult for many patients to provide. The instructor should point out that insights from the hypothesis generating interviews (e.g., about common foods) are most likely to result if one person does all of the interviews.

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.

- *The cases are spread over 10 counties. Patients interviewed did not report attendance at any common event. This suggests that the source is relatively widely distributed within the state.*

- *Onset of symptoms among known cases extends over approximately one month. This suggests that the source of contamination is a product with an appreciable shelf-life or ongoing production of a contaminated product. (Or it could indicate secondary spread.)*

- *The median age of patients is 31 years (range 2-76); 68% of cases are among females. From surveillance data, diarrheal and foodborne diseases are more commonly reported among younger children with a slight predominance among males in the older age groups. The age/gender distribution in this outbreak is slightly atypical but is similar to that seen in outbreaks of Salmonella sp. caused by salad items and sprouts.*

- *Although ground beef is the most common source of E. coli* O157:H7 *infection in the United States, non-meat items (e.g., lettuce, apple cider, unpasteurized apple juice) have been implicated in other outbreaks. Furthermore, consumption of raw alfalfa sprouts has been associated with outbreaks due to various serotypes of Salmonella.*

LEADING HYPOTHESIS: lettuce or sprouts

[NOTE: At the time that this outbreak investigation occurred, alfalfa sprouts had never been implicated as a source of E. coli O157:H7. As a result, the investigators proceeded with caution in exploring this 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?

Controls should be individuals without the disease in question who are representative of the population from which the cases originated. In addition:

- *controls should be at risk for the disease (i.e., they can develop the disease)*
- *controls (and cases) should have the potential for exposure to the risk factor of interest, and*
- *selection of controls (and cases) should be independent of their exposure status*

In this outbreak, controls should be from the same communities as the cases. Controls should not have had symptoms suggestive of E. coli O157:H7 *infection (i.e., diarrhea consisting of ≥3 unformed stools per day or bloody diarrhea) during the outbreak period.*

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

Matching generally refers to a case-control study design in which controls are intentionally selected to be similar to cases on one or more characteristics. The characteristics most appropriately specified for matching are those which are potential confounders of the exposure-disease association of interest (e.g., age, gender, geographic area). By matching, the distribution of the selected characteristics will be identical among cases and controls and, therefore, will be eliminated as potential confounders in the analysis. Special methods must be used to analyze study results if cases and controls are matched on some characteristic.

Given the unique age and gender distribution of cases, it might be a good idea to match on these characteristics in the study design, but there are arguments for and against matching.

Advantages of matching:

- *Matching on factors such as neighborhood, friendship, or sibship (or even age and gender) may control for confounding by numerous social factors that would be otherwise impossible to measure and control.*

- *Matching may be cost- and time-efficient, facilitating enrollment of controls, when the control knows the case and, therefore, is more likely to participate.*
- *Appropriate matching increases statistical efficiency of an analysis and, thus, provides narrower confidence intervals.*

Disadvantages of matching:
- *Matching on a factor prevents one from examining its association with disease.*
- *Matching may be cost- and time-inefficient, if considerable work must be performed to identify appropriately matched controls.*
- *Matching on a factor that is not a confounder or having to discard cases because suitable controls could not be found decreases statistical efficiency and results in wider confidence intervals (i.e., decreases precision).*
- *Matching complicates analyses, particularly if confounders are present.*

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

Often it is difficult to know who the controls should be. Practical matters need to be taken into consideration, such as how to contact potential controls, gain their permission, ensure that they are free of the disease under investigation, and get appropriate exposure data from them. The following methods have been used to identify controls in different settings:

- *Random digit dialing (i.e., random selection of either all seven digits or the last four matching on the associated case's telephone exchange) and sequential digit dialing (i.e., patient's telephone number + 1)*
  Advantages:
  - *limited assistance needed from cases (don't need to ask them to identify potential controls)*
  - *may produce controls that are more representative of the community from which cases came than physician-matched controls, neighborhood controls, or friends and acquaintances of patients (i.e., a less biased sample)*

  Disadvantages:
  - *possibility of reaching many disconnected and commercial telephone lines (which cannot provide controls) (NOTE: This problem decreases somewhat with sequential digit dialing.)*
  - *may require many calls before an appropriate age and gender-matched control is identified*
  - *low participation rate among potential controls*

- *Neighborhood controls (selected either by home visits or using a reverse telephone directory which provides the telephone number for a particular address)*
  Advantages:
  - *opportunity for similar exposures among cases and controls*
  - *increased likelihood that potential controls will participate due to interest in threats to own community*

  Disadvantages:
  - *difficulty finding age and gender-matched controls for some cases, particularly those at the age extremes or living in rural areas*
  - *potential for overmatching (e.g., if neighbors share a product or have common use patterns, there is an increased likelihood that the study will fail to implicate a source)*
  - *because most people are not at home during the day it requires investigators to work at night*
  - *requires an enormous logistical effort in a multicommunity outbreak*

- *Ask patient or patient's family to name person(s) of the same age group and gender*
  Advantages:
  - *easy for investigator to locate potential controls*
  - *increased likelihood that potential controls will participate due to interest in threats to own community or friendship with case*
  - *opportunity for similar exposures among cases and controls*

  Disadvantages:
  - *potential for overmatching (e.g., if friends share a product or have common use patterns, there is an increased likelihood that the study will fail to implicate a source)*
  - *greater care must be taken to prevent a breach of case confidentiality*

- *Other patients seen by the same physicians as the cases (remember, all cases were culture confirmed and, therefore, probably seen by a physician)*
  Advantages:
  - *may a be less biased sample than neighborhood controls or friends and acquaintances of patients*

  Disadvantages:
  - *loss of representativeness of the community because persons going to the doctor may be ill and not reflect general population characteristics and behaviors*
  - *difficulty finding age and gender-matched controls for some cases, particularly those at the age extremes*

- *someone in the physician's office needs to be instructed on the appropriate selection of controls*
- *requires an enormous logistical effort in a multicommunity outbreak*

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

Considering the incubation period (i.e., range from 3-8 days), it would be advisable to collect information from cases on exposures that occurred in the week before onset of E. coli O157:H7 symptoms. (NOTE: A week as opposed to 8 days will be easier for study subjects to consider.)

Ideally, for controls, one would collect information from the same dates as the matching case. If a great deal of time has passed since the case became ill, however, this might be a difficult task (more so for controls than cases because there is no illness to spur their memories). In these instances, one might collect information from the week before the study or ask the individual what they would have normally consumed in a week during the month that the case became ill. Many times investigators will use more than one time frame in the collection of exposure information.

(NOTE: Although a lot of effort may be put into identifying specific time frames for exposures, in practice, it is likely that one gets part recall and part preference data. By asking specific information about sources [e.g., "Where did you eat that?", "What was the brand?", "At what store did you purchase that?"], however, investigators can help stimulate the memory of study subjects.)

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?

Students should consider the following possible explanations in evaluating the elevated odds ratio for consumption of sprouts:

- *chance*
- *selection bias (e.g., persons with reported exposures to alfalfa sprouts were more likely to be cultured for and diagnosed as having E. coli O157:H7 infection than persons who did not report this exposure to their physician)**
- *information bias (e.g., cases were more likely to remember they had eaten alfalfa sprouts than controls)*
- *confounding (e.g., eating alfalfa sprouts was associated with some other characteristic that was truly associated with the disease such as eating lettuce in a salad)*
- *true association*

**Because this was the first E. coli O157:H7 outbreak associated with alfalfa sprouts, selection bias based on exposure to sprouts may have been unlikely.*

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?

It is possible that these cases ate sprouts and forgot they ate them or ate them inadvertently. It is also possible that the sprouts cross-contaminated other food items that the cases did eat such as other sandwich or salad ingredients or that the sprouts and some other food item were contaminated through the same source (e.g., contaminated rinse water). Another explanation is that the incubation period for the 12 cases was longer than 7 days and they ate the sprouts before the period of interest for the case-control study. Another possibility is that non-sprout eating cases were secondary cases in this outbreak and became infected through person-to-person transmission. Finally, it is possible that the sprouts association is not a true association and some other explanation exists for all of the cases.

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.)

To take action, one must decide if there is enough evidence to implicate the alfalfa sprouts and/or sufficient information on which to take action. Although the investigators had solid epidemiologic evidence to implicate the alfalfa sprouts at this point, the main difficulty with taking action was insufficient data to recall a product such as a lot number or sprouter. So, investigators decided additional studies were necessary.

Desirable studies include culturing of the implicated sprouts; a traceback of the sprouts to the distributor, processor, and producer; examination of the chain of production of the sprouts from the farm to the table; and applied research on alfalfa sprouts and survival/growth of E. coli O157:H7 (e.g., the ability of E. coli to survive and grow on alfalfa seeds/sprouts at each step of the production process). In addition, a closer examination of the 12 cases that reported not eating alfalfa sprouts might be helpful in identifying other routes of infection.

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?

The following criteria should be considered in deciding to undertake a full traceback procedure in a foodborne disease outbreak investigation:

- *Is there solid epidemiologic evidence linking the outbreak and the implicated product? (e.g., Was a controlled study undertaken? Was the selection of subjects and collection of information unbiased? Could confounding account for the association? How likely is it that chance could account for the elevated measure of association?)*
- *Has onsite mishandling or environmental contamination of the product been evaluated?*
- *Could the product be commercially distributed in a way that is consistent with the outbreak?*
- *What is the scope of the outbreak? Are the cases in the outbreak from a number of different geographic locations (e.g., different cities, counties, or states)? Is it possible that the implicated product could be causing other cases elsewhere?*
- *Is there historical precedence for the product being contaminated with this organism (or with a similar organism)?*
- *Is there microbiologic evidence of a linkage between the outbreak and the implicated products? (e.g., Has the outbreak organism been isolated from the implicated product with similar subtyping results?)*

It is likely that one would want to do a traceback of the alfalfa sprouts implicated in this investigation for the following reasons:

- *From the case-control study, there is strong epidemiologic evidence linking alfalfa sprouts to the outbreak.*
- *Because the cases came from at least 10 different counties in one state (and, as will be noted later, also came from other states), it seems unlikely that contamination of the sprouts occurred in the homes of the cases or individually in all the stores where they were purchased.*
- *Because the outbreak spreads over a wide geographic location, the implications are broader and more persons are likely to be at risk.*
- *At the time of this outbreak, alfalfa sprouts had not previously been implicated in the transmission of E. coli O157:H7; however, several outbreaks of Salmonella sp. had been associated with consumption of seed sprouts making it biologically plausible.*

NOTE: In this investigation, a traceback was undertaken only after a food item was implicated. In some investigations, however, the traceback may occur earlier in the investigation (as part of the hypothesis generating interviews or the case-control study) and provide epidemiologic information that may be critical to implicating a product in the first place. A good example might be the consumption of a relatively common food item such as chicken. Consumption of chicken may not be more common among cases than controls but consumption of a certain chicken product or brand may be. Only by collecting detailed product information (i.e., similar to that collected for a traceback) can such an association be detected.

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?

A full traceback of an implicated food item usually begins with information available at the time of purchase by the consumer and extends back to the very beginning of its production. All steps from harvest to consumption should be considered.

One usually begins with the consumer or the establishment where the food item was served and the collection of information on where and when the food item was purchased. Leftover packaging from the implicated product can be helpful in tracking down the manufacturer or distributor of the food item; however, packaging is not always available, especially for tracebacks of fresh produce.

The next step is usually the store where the food item was purchased. Here one wants to look for the most likely source of the food item during the time that the case or the food establishment purchased it (not just the usual sources for the store). Store purchase records, invoices, and inventories can then be used to work back up the distribution chain.

The following product information is helpful to the traceback procedure:

1) complete product name (including brand name)
2) size/weight of package or container and type of packaging
3) code numbers
4) lot numbers
5) sell by dates
6) expiration dates
7) manufacturer (and address)
8) wholesalers (and addresses)
9) distributors (and addresses)
10) dates shipped/received/purchased

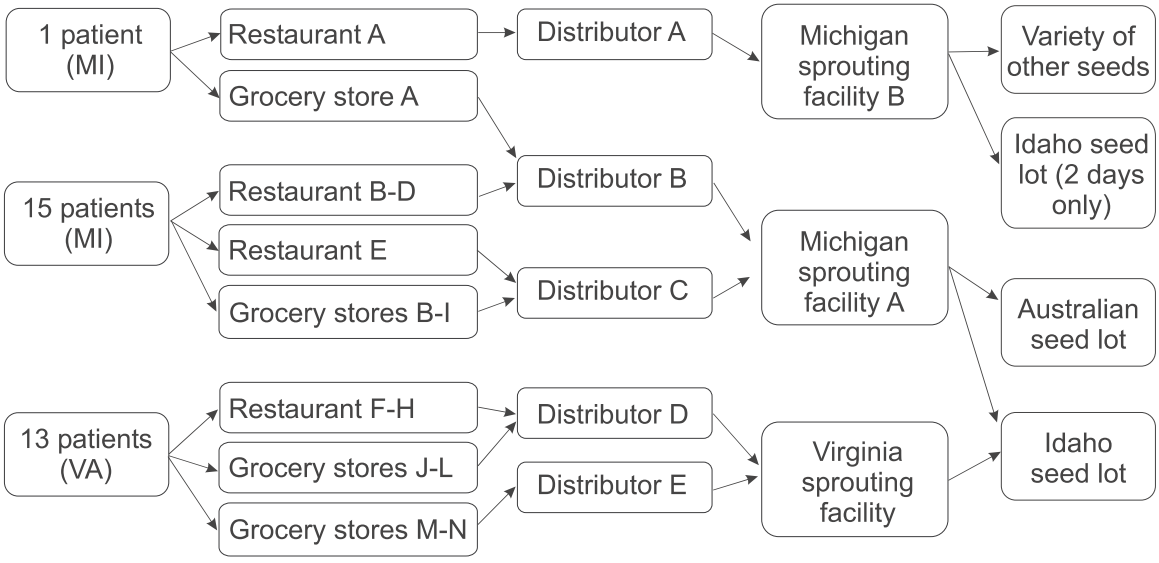
The last four items on this list are critical. In addition, it is important to clarify what "dates" on invoices and other records represent (i.e., the date shipped? received? purchased?) so that the investigation and further search can be adjusted accordingly.

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?

Because two sprouting facilities (in two states) were associated with the implicated alfalfa sprouts and a single lot of seeds (from Idaho) were common to both, it seems likely that contamination of the seeds occurred before sprouting. Possible points of contamination include in the field (during growth or while being harvested), during processing of the seed, while in storage, or during transport. Further information needs to be collected on each of these steps to help identify the most likely place of contamination.

(NOTE: The distribution chain in Figure 4 is largely fictitious and was simplified to avoid confusion. Grocery stores and restaurants often have more than one supplier during a particular time period, greatly increasing the complexity of the traceback.)

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?

Although cattle are the primary reservoir for E. coli O157:H7, other animals (e.g., deer and elk) can also be carriers or become infected. One would probably want to examine the following to determine if they might allow direct contact between the alfalfa seed and any of these animals or their feces:

- *site of the fields (e.g., presence of animals and/or their feces, relationship to animal pastures, whether fields are enclosed by fences to restrict access, slope and direction of run-off)*
- *soil, fertilizer, pesticides*
- *irrigation water (including inspection of source wells)*
- *harvesting equipment*
- *processing equipment (e.g., equipment used for washing or bagging)*
- *storage containers*
- *storage rooms*
- *shipping trucks*

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?

One needs to consider 1) the immediate problem with this implicated lot of seed and 2) the larger issue of seed sprouts as vehicles for pathogenic agents.

For the immediate problem, all remaining seeds and alfalfa sprouts from the implicated lot should be removed from the market. Persons who have purchased sprouts from the implicated lot should be instructed to destroy any remaining sprouts or return them to the store at which they were purchased. One might want to examine other lots of seed used for sprouting from these same producers. If there is evidence for fecal contamination, other lots intended for human consumption should also be removed from the market. The producers of these particular seeds should be informed of the need to protect alfalfa and other seeds used in sprouting from contamination during growing, harvesting, and packing. Specific sources of contamination should be identified and eliminated from these growing sites.

*Addressing seed sprouts as a high risk vehicle for foodborne diseases is a more difficult task. Although this study was the first to implicate alfalfa sprouts as the vehicle for E. coli O157:H7, alfalfa and other sprouts had been linked to the transmission of Salmonella sp. and radish sprouts had been associated with an outbreak of E. coli O157:H7.**

Sprouts appear to be at increased risk for contamination with foodborne pathogens for the following reason:

- *The seeds used to produce sprouts are a natural product, grown in the dirt, surrounded by dirt and the associated feces and bacteria. Most seeds are destined only to grow more alfalfa (i.e., to provide hay to feed cattle); less than 0.5% of the total production is used by sprouters. The latter are not typically grown under special conditions (e.g., "feces free" fields) but rather are diverted from the other lots intended for alfalfa hay production. Therefore, the risk of contamination of the seeds is high.*
- *To enable sprouting, the seeds are kept moist and warm for >24 hours. (See Appendix 3.) This allows multiplication of any bacteria by as much as 4 to 6 logs of growth, further increasing the risk of contamination.*
- *Efforts to decontaminate the seeds before sprouting have not been successful. Exposure to a chlorine solution decreases contamination a thousand fold. But this is probably insufficient to remove the potentially high level of contaminants occurring in some instances. Radiation eliminates bacterial contamination but reduces germination rates of the seed and is unacceptable to some consumers.*

Given the nature of sprout production and the current inability to eliminate contamination, not many options remain:

- *Continue applied research to find ways to successfully decontaminate the seeds/sprouts.*
- *Educate sprout growers on appropriate growing conditions and handling of sprouts to limit contamination.*
- *Educate the public about the riskiness of sprouts and suggest that persons at high risk for complications of infection (e.g, children <5 years of age, immunocompromised individuals, and the elderly) avoid consuming sprouts.*

- *Require sprout producers to label sprouts as risky foods, and suggest that if people want to avoid the risk of diarrheal illness, including potentially fatal E. coli O157 H7 infection, they should avoid consuming them.*
- *Remove sprouts from the market for human consumption until their safety can be assured.*

**A 1988 outbreak of Salmonella Saint Paul infections in Europe was linked to mung bean sprouts. A small 1990 cluster of Salmonella Anatum infections in the United States was suspected to be linked to one grower's alfalfa sprouts, but the source of contamination was not determined. A 1994 Salmonella Bovismorbificans outbreak in Finland and Sweden was traced to Australian alfalfa seed. In 1995, it was concluded that sprouts caused an international outbreak of Salmonella Stanley, affecting persons in more than 17 states in the United States and Finland. In that same year, another multinational outbreak of salmonellosis (due to S. enterica serotype Newport) was linked to alfalfa seeds after an increase in infections was detected in Oregon and British Columbia. In 1996, almost 10,000 cases of E. coli O157:H7 occurred among school children in Japan. The outbreak was ultimately shown to be caused by radish sprouts grown from seed imported from the U.S.*

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?

Changes in product processing (e.g., aseptic seed production methods, irradiation of seeds), if possible, are likely to be the most effective form of intervention in this instance. The other two require behavioral and attitudinal changes among a large number of individuals. Behaviors and attitudes can be changed but necessitate broad-reaching, motivating, and culturally appropriate health education. In addition, education must continue indefinitely as each new generation of consumers (and growers/producers) arrives on the scene. Successes in public or producer education are known but require continuing resources and high levels of public interest in the problem. Examples include E. coli O157:H7 in hamburger, Salmonella sp. in chicken, and Trichinella sp. in pork.

*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)

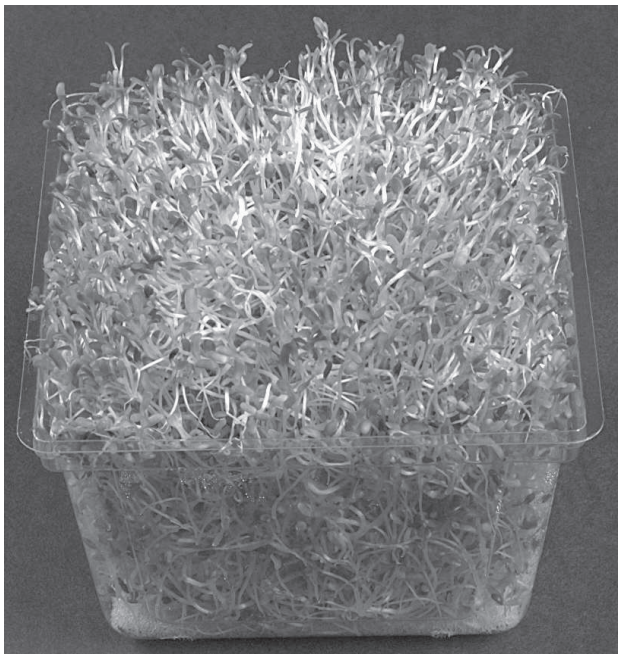
OTHER SPECIAL EVENTS: square dancing at senior citizens center (no food served)

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.