PROCEDURES TO INVESTIGATE FOODBORNE ILLNESS

Fifth Edition — 1999

Published by the

International Association for Food Protection

Reprinted 2004

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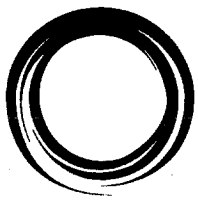

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PROCEDURES TO INVESTIGATE FOODBORNE ILLNESS

Fifth Edition 1999

(Reprinted 2004)

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FOREWORD

This fifth edition of *Procedures to Investigate Foodborne Illness* is designed to guide public health personnel or teams in any country that investigate reports of alleged foodborne illnesses. It is companion to two other manuals published by the IAFP, *Procedures to Investigate Waterborne Illness* and *Procedures to Investigate Arthropod- and Rodent-borne Illness.* These three manuals are based on epidemiologic principles and investigative techniques that have been found effective in determining causal factors of disease incidence.

This edition describes procedures to:

- Handle illness alerts and food-related complaints that may be related to illness
- Interview ill persons, those at risk, and controls
- Develop a case definition
- Collect and ship specimens and samples
- Conduct hazard analysis at sites where foods responsible for outbreaks were produced, processed or prepared
- Trace sources of contamination
- Identify factors responsible for contamination, survival of pathogenic microorganisms or toxic substances, and/or propagation of pathogens
- Collate and interpret collected data
- Report information about the outbreak.

These guidelines are presented in the sequence usually followed during investigations. They are organized so that an investigator can easily find the information needed in any phase of an investigation. The instructions, forms, tables, and information on selected etiologic agents, diseases, and food vehicles are comprehensive but not exhaustive.

This manual combines the principles and techniques of epidemiology, statistics, and hazard analysis that guide the formation of rational hypotheses and their testing. The Table of Contents serves as an outline, and a flow diagram shows interrelationships of the activities and their typical sequence in an investigation. This edition expands techniques to carry out on-site hazard analyses during outbreak investigations. It also includes revised and expanded keys to detect factors that likely contributed to contamination, survival, and/or increase of biological or chemical etiologic agents for a variety of foods that have been processed or prepared in different ways. These keys guide investigators to look for events that most likely contributed to the outbreak and, thereby, to indicate action that must be taken to control the situation and to prevent recurrence. The *Analyze Data* section has been expanded to include more statistical calculations. Forms have been modified to improve the quality of investigations. The listing of equipment useful for investigations has been expanded to include equipment that has applications for conducting hazard analyses and statistical calculations as well as collecting samples and specimens. The listing of illnesses attributed to foods and the references have been updated.

The final foodborne illness summary report form contains detailed categories of places foods were acquired; the sites of contamination, survival and

propagation, which may be at different locations; method of processing or preparation; factors that contributed to the outbreak; and space for a narrative summary. Collection of these sorts of data, over time, can provide focus for preventive and control measures.

The forms have been reduced to fit the pocket size of the manual. They can be expanded for use on 8½ × 11-inch paper by increasing the size by approximately 130 times during photocopying. Summary and data tabular forms can be increased to fit on 11 × 17-inch paper. The office of the Internation Association for Food Protection has an 8½ × 11-inch set of forms available.

Kits including the manual, expanded-size forms and equipment useful for investigation can be pre-assembled so that upon notification of an outbreak, investigation can be initiated without delay.

This manual supersedes previous editions. It provides information to conduct thorough investigation of outbreaks of foodborne diseases.

INTRODUCTION

During production, harvesting, processing, packaging, transportation, preparation, storage, and service, any food may be exposed to contamination with poisonous substances or infectious or toxigenic microorganisms. Processing or preparation failure may lead to survival of microorganisms or toxins, and time-temperature abuse can allow proliferation of pathogenic bacteria and molds. In addition, some plants are intrinsically toxic. Animals may acquire toxins from their food or metabolize them, or they become infected with or colonized by pathogenic bacteria, viruses, and parasites. If a product contaminated with sufficient quantities of poisonous substances or pathogenic microorganisms is eaten, foodborne illness will result in susceptible persons.

Foodborne illnesses comprise the various acute syndromes that result from ingestion of contaminated foods. They are classified as:

- Intoxications caused by ingestion of foods containing either poisonous chemicals or toxins produced by microorganisms
- Toxin-mediated infections caused by bacteria that produce enterotoxins (toxins that affect water, glucose, and electrolyte transfer) during their colonization and growth in the intestinal tract
- Infections caused when microorganisms invade and multiply in the intestinal mucosa or other tissues.

Manifestations range from slight discomfort to acute illness to severe reactions thay may terminate in death or chronic sequelae, depending upon the nature of the causative agent, number of pathogenic microorganisms or concentration of poisonous substances ingested, and host susceptibility and reaction.

The public relies on the food industry as well as health and food regulatory officials for protection from foodborne illness. Such protection depends upon rapid detection of outbreaks and a thorough knowledge of the agents and factors responsible for foodborne illness. Current food distribution systems are able to move contaminated products throughout a country or to any place in the world within days of processing. Local investigations of reported foodborne illness can, therefore, improve the surveillance and control of foodborne disease by rapid reporting to the national and international levels.

The purposes of a foodborne illness investigation are to:

- Identify illnesses associated with an incident and verify that the causative agent is foodborne
- Detect all cases, the causative agent, the implicated food(s), and the place(s) where food was mishandled or mistreated
- Determine source and mode of contamination, processes or practices by which proliferation, and/or survival of the etiologic agent occurred
- Stop the outbreak or prevent further exposures
- Gather information on the epidemiology of foodborne diseases and the etiology of the causative agents that can be used for education, training, and program planning, which can make an impact on preventing foodborne illness.

Once the responsible food has been identified, further cases can often be prevented by halting its distribution and sale; recalling production lots already distributed; and quarantining, reprocessing, or disposing of the contaminated foods to prevent their entry or reentry into food channels.

As epidemiologic data accumulate, information will indicate critical control points in food production, processing, and preparation as well as appropriate methods for controlling and preventing foodborne disease. This information will guide administrators in making rational decisions and setting program priorities to provide the highest degree of food safety at the lowest cost.

A flow chart, *Sequence of events in investigating a typical outbreak of foodborne illness,* (Figure A, page 142) shows the sequential steps, as presented in this manual, in investigating a typical outbreak of foodborne illness and indicates their relationships. A description of each step is given in this manual.

DEVELOP A FOODBORNE DISEASE SURVEILLANCE SYSTEM

An effective surveillance system will:

- Detect illness and outbreaks
- Investigate suspected outbreaks or clusters of illnesses
- Collate and interpret data
- Disseminate outbreak reports and surveillance summaries to appropriate agencies.

Many types of disease-detection and disease-reporting systems may already exist at the local or state/provincial level and can be incorporated into a foodborne disease surveillance program. These include:

- Mandatory (or voluntary) laboratory- or physician-based reporting of specific infectious diseases
- Hospital emergency and urgent-care clinic records
- Public complaints made to health agencies
- School illness and absentee records
- Absentee records of major employers
- Sentinel studies of selected diseases
- Sales of antidiarrheal drugs.

Organize the System and Develop Procedures

An effective foodborne disease surveillance system requires close cooperation between key personnel in public and private health agencies. When your agency contemplates developing or improving a foodborne disease surveillance program, give top priority to obtaining financial and administrative support. Then, identify a key person to create, implement, and manage the system. This person takes responsibility to:

- Review existing reporting systems that could be incorporated into the foodborne disease surveillance system

- Identify the types of information that cannot be obtained from existing reporting systems but that need to be collected or addressed by the foodborne disease surveillance system
- Identify ways to merge or integrate the data collected by existing systems with data gathered in the foodborne disease surveillance system
- Identify collaborating agencies and staff
- Train agency staff in surveillance methods
- Assemble materials that will be required during an outbreak investigation
- Evaluate the effectiveness of the system.

Develop procedures to seek and record complaints about foodborne diseases. For example, list the telephone number of the foodborne disease (food poisoning) unit prominently under the appropriate health agency in the phone book or on the front inside cover. To be most effective, have this number monitored 24 hours a day, 7 days a week either by an answering service or a 911 emergency-services system. Identify medical-care facilities and practitioners, and seek their participation. Direct education activities, such as newsletters and talks at meetings, to stimulate participation in the program. Encourage food and tourist industries to report complaints of suspected foodborne illness. Also, encourage private and hospital laboratories to report isolations of bacteria (e.g., *Escherichia coli* O157:H7, *Salmonella, Shigella, Vibrio cholerae*), viruses (e.g., small round structured viruses and hepatitis A virus), parasitic agents (e.g., *Trichinella spiralis, Cyclospora cayetanensis, Giardia lamblia*), and other agents that may be foodborne. Develop a protocol for coordination with agencies that might cooperate in investigation activities, including 24-hour, 7-day-a-week contacts. Coordinate with state/provincial, district and national agencies that have surveillance and food regulatory responsibilities, and other national and international health agencies, as appropriate.

Assign Responsibility

Delegate responsibility to a professionally-trained person who is familiar with epidemiologic methods and food safety to direct the surveillance program, take charge when foodborne and enteric disease outbreaks are suspected, and handle publicity during outbreaks. Delegate responsibility to others who will carry out specific epidemiologic, laboratory, and on-site investigations.

Establish an Investigating Team

Establish a team of epidemiologists, sanitarians, public health inspectors, microbiologists, nurses, physicians, public information specialists, and others (e.g. toxicologists) as needed. Free flow of information and coordination are essential among those participating in foodborne and enteric disease surveillance and investigation, particularly when several different agencies are involved. Food-related complaints are usually directed to health departments or food regulatory agencies, but perhaps to different jurisdictions. Therefore, it

is essential that these complaints be recorded by the agency receiving the alerts. Whenever possible, share the information with participating parties or nearby jurisdictions by rapid means such as electronic mail or fax. Give top priority to illness and outbreak investigations.

Train Staff

Select staff members to participate in the foodborne disease surveillance program based on their interest, education, and ability. Inform them of the objectives and protocol of the program. Emphasize the value of disease surveillance in a food safety program. Assign them to investigations, and to use epidemiologic information and approaches in their routine disease surveillance and prevention activities. Develop their skills so that they can carry out their role effectively during an investigation and teach them procedures to interpret data collected during investigations. Conduct seminars routinely, and during or after investigations, to update staff and keep agency personnel informed. Train office workers who receive calls concerning foodborne illnesses to give appropriate instructions. Those who participate in the investigation will learn from the experience and often are in a position to implement improvements after the investigation is completed.

Assemble Materials

Assemble and have readily available kits with forms and equipment as specified in Table A, *Equipment useful for investigations* (page 98). Restock and maintain kits on a schedule recommended by laboratory staff to ensure their stability and sterility. Assemble a reference library, including e-mail and internet addresses, on foodborne and enteric illnesses and control measures; make it available to the staff. Update this library with information on foodborne pathogens and investigation techniques from articles in scientific journals, new books, manuals, and internet searches. (See Further Reading, page 69 for suggestions.)

INVESTIGATE OUTBREAKS

Investigating an outbreak involves:

- Receiving notification of illnesses that might be foodborne
- Interviewing ill persons and persons at risk who remained well
- Making epidemiologic associations from initial information
- Forming hypotheses.

Further investigation to confirm or refute the hypotheses includes:

- Collecting clinical specimens and food samples
- Conducting an on-site investigation to determine the source and mode of contamination, survival, and/or proliferation of the etiologic agent.

Act on Notification of Illness

Prompt handling and referral of food-related complaints, rapid recognition of the problem, and prevention of further illnesses are the foundations of

a successful investigation. This first contact with the public is a vital aspect of an investigation.

Receive complaints or alerts

Upon receiving a complaint or an alert about a food or illness potentially attributed to food, record the information on Form A, *Foodborne, waterborne, enteric illness report* (page 72). An alert or complaint relating to food may involve foodborne illness, food spoilage, adulteration of a product, mislabeling, or an unsanitary establishment. Alerts also can be initiated by reports from physicians, by records of foodborne pathogens isolated by laboratories, by calls to poison control centers, and by reports of treatment given in either hospital or private emergency rooms, or by emergency squads. The form provides information upon which to decide whether an incident should be investigated. It is not difficult to fill out and can be completed by a public health professional or trained office worker.

Assign a sequential number to each complaint. If additional space is needed to record information, use the reverse side or attach additional sheets. Always ask the complainant to provide names of other persons at the event under suspicion, whether or not ill, and names of any other persons who are known to be ill with the same syndrome. Follow up by contacting these additional persons.

It is of paramount importance to collect clinical specimens and a sample of the suspect food or water as soon as possible. Tell the complainant to collect stool or vomitus specimens from ill persons in a clean jar or plastic bag and to seal tightly and label clearly with name of ill person and date. Then, put all specimen containers into either a paper or plastic bag, label, and store in a refrigerator (but do not freeze). Emphasize the need to retain a sample of all suspect foods in their original container or package, if practical. Otherwise, instruct this person to put a half pint (250 g/ml), if that much is available, or otherwise all of the remaining food, into a clean container that has been boiled or into an unused plastic bag. Also, put this jar or bag into either a paper or plastic bag, label with name of food and date, and refrigerate but do not freeze. (See section *Obtain clinical specimens,* page 13, and *Collect samples of suspect foods,* page 36, for detailed procedures.) Tell this person to hold all clinical specimens and food samples until the health agency evaluates the epidemiological evidence and arranges, if necessary, to collect them. If it is determined that the specimen or sample is not necessary, notify the complainant and advise on proper disposal of the material. Collect clinical specimens and food samples as soon as practicable and according to the recommendations given in this text.

If there is a cluster of cases, monitor reports from physicians, complaints about food, or records of laboratory isolation of enteric pathogens that may suggest outbreaks of disease or contributory situations.

Log alert and complaint data

Extract key information (see * and † entries) from Form A and enter it onto Form B *Foodborne, waterborne, enteric illnesses log* (page 73). See Table 1 (page 6–7) for an example.

Table 1. *Foodborne, waterborne, enteric illnesses log*

Complaint			Illnesses			Food		Water				
No.	Date	Type[1]	Onset date	No. ill	Predominant symptoms/ signs	Alleged/ Suspected	Where eaten within 72 h	Where ingested within 2 wk	Where contacted within 2 wk	Source[2]	History of exposure[3]	Comments
101	8/16	I	8/15	1	Diarrhea			Redguard		C	IT	*G. lamblia* isolated from stool specimen
102	8/20	I/UE	8/18	1	Diarrhea	Hamburger	Speedy Foods	Dixon		C		
						Roast beef	Nitestar Club	Jonesville		C		
103	8/21	I	8/21	12	Diarrhea		Dixon Day Care Center	Dixon		C	DC	
104	8/23	CF		0		Corn		Daulton				Swollen can, Brand W, code LM 308
105	8/24	I	8/23	1	Vomiting	Ham	Joe's Diner	Dixon		C		
106	8/23	I	8/23	1	Nausea, vomiting	Cold cuts from deli	Jo's Market	Dixon				
107	8/26	I	8/24	1	Diarrhea, fever, vomiting			Plainville		W		*S. chester* isolated from stool
108	8/26	I	8/25	1	Diarrhea, fever		Church supper	Dixon		C		
109	8/27	I	8/25	1	Diarrhea, chills			Midvale				*S. anatum* isolated from stool

Table 1. *Continued*

Complaint			Illnesses			Food		Water				
No.	Date	Type[1]	Onset date	No. ill	Predominant symptoms/ signs	Alleged/ Suspected	Where eaten within 72 h	Where ingested within 2 wk	Where contacted within 2 wk	Source[2]	History of exposure[3]	Comments
110	8/27	I	8/26	1	Vomiting, diarrhea	Oysters	Fred's Cafe	Dixon		C		
111	8/29	I	8/28	1	Vomiting		Joe's Diner	Clear Falls		W		
112	8/29	CF		0		Baby cereal		Dixon		C		Metal fragments found in Brand X, Lot JK201E
113	8/30	I	8/28	1	Nausea, vomiting, headache	Oysters	Ralph's Seafood	Dixon		C		Canned by Food Processors Inc. Lot L36105F
114	9/1	I	8/29	1	Diarrhea, fever, vomiting		Dixon Arts Festival	Dixon		C		*S. chester* isolated from stool
115	9/2	I	8/29	1	Blurred & double vision, paralysis			Dixon		C		Hospital reported assisted breathing

[1]Type complaint: I = illness; CF = contaminated/adulterated/spoiled food; UE = unsanitary food establishment; DW = poor quality drinking water; RW = poor quality recreation water; MP = Complaint related to media publicity; D = disasters.
[2]Water source: C = community; NC = non-community; W = well; B = bottled; S/L = stream/lake.
[3]Exposure history: DT = domestic travel (out of town, within country); IT = international travel; DC = Day care; CI = contact with ill person outside household or visitor to household; An = exposure to ill animal.

Record time of onset of the first symptom or sign of illness, number of persons who became ill, predominant symptoms and signs, and name of the food alleged to have caused the illness, if stated. Also, enter names of places or common gatherings at which the stricken person ate during the 72 h before onset of illness and other pertinent information. Under "history of exposures" column, use appropriate abbreviations to indicate the reported information. Under "comment", enter notations of type of agent isolated, results of specimen tests, places where food or water was consumed during travel, names and locations of restaurants or other foodservice facilities, and other pertinent information. At this phase of the investigation, it probably will not be known whether the illness is foodborne, waterborne, or spread person-to-person. This log can be kept either in a book or in a computer (e.g., Epi Tracker software).

Interpretation of Table 1:

- Entry 101—Get further details on the patient's symptoms and seek other cases. The report of foreign travel suggests an infection that may have been acquired outside the country. Follow-up of such cases may identify an outbreak of international scope. If so, inform state/provincial and national authorities concerned with surveillance of foodborne disease about the situation.
- Entry 102—Possibly foodborne but could be food from either Speedy Foods, Nitestar Club or elsewhere; look for and log possible cases with time/place associations. (See section *Make time, place and/or person associations,* page 17.)
- Entry 103—Initiate investigation; 12 cases suggest an outbreak that has a common time/place association.
- Entry 104—Refer complaint to agency or department concerned with food quality.
- Entry 105—The vomiting suggests an illness of short duration. There may be an association with the meal eaten prior to vomiting.
- Entry 106—The syndrome is similar to that of Entry 105. The names Joe's and Jo's are similar; clarify.
- Entry 107—Look for other cases having the same infection.
- Entry 108—Look for other cases with similar signs and symptoms with the same place/event association.
- Entry 109—A second isolation of *Salmonella* within a few days, but a different serotype. Association with Entry 107 is unlikely.
- Entry 110—The illness is similar to that caused by Norwalk-like viruses, which is commonly transmitted by oysters. Look for other cases with similar signs or with a place association.
- Entry 111—The second complaint associated with eating at Joe's Diner is a possible common place association (see Entry 105); investigate.
- Entry 112—Report situation to agency or department responsible for regulating food processing and monitoring adulterated food.
- Entry 113—This syndrome also suggests Norwalk-like illness. There may be a possible association with the place where the oysters were harvested and there may be an association with Entry 110; investigate.

- Entry 114—This illness may be associated with Entry 107. Both persons are ill with salmonellosis caused by the same serotype of *Salmonella, S. chester*; investigate.
- Entry 115—The signs and systems suggest botulism; investigate immediately and collect clinical specimens and food samples.

Review the log each time an entry is made and also each week to discover clusters of cases and/or involvement of a common food or place of eating that might otherwise go undetected. If your agency has district offices or if there are nearby jurisdictions (as in metropolitan areas), periodically send copies of log sheets to a central coordinating office (e.g., weekly or when there are 10 to 20 entries). Reports of current illness levels should include historical information on illness trends in the community so that new data can be considered in the appropriate context. Report to your supervisor if you suspect any time, place, or person associations and take steps to initiate an investigation (see page 17).

Refer complaint to proper agency

Refer complaints that fall outside your agency's range of operations to the appropriate authority and indicate the action taken in the disposition box on Form A. Develop a working relationship with such authorities so they will reciprocate in situations that may be associated with illness. Often an investigation requires efforts of more than one agency. Cooperation and prompt exchange of information between agencies are vital.

Prepare for the Investigation

Assign responsibility for an investigation to the person heading the investigative team if this was not done when the surveillance protocol was established. Delegate sufficient authority and provide resources to this person so that the task of investigation can be accomplished. Inform everyone working on the investigation that findings are to be reported to this person.

Before beginning the investigation, check the supply of forms and the availability of equipment suggested in Table A, *Equipment useful for investigations* (page 98). Obtain any needed materials or additional equipment.

If the alert or complaint suggests a possible outbreak, inform laboratory personnel of the type of outbreak and estimated quantity and arrival time of clinical specimens and food samples to be collected. This information will give laboratory managers time to prepare media and allocate personnel. Consult laboratory personnel about proper methods for collecting, preserving, and shipping food samples and clinical specimens if such information is needed. Get appropriate specimen containers from them.

Verify Diagnosis

An ill person or family member, physician, or hospital staff member may report suspected cases of foodborne illness. Whatever the source of the report, verify the diagnosis by taking a thorough case history and, if possible, by reviewing clinical information and laboratory findings. (This analysis can be

further substantiated by detecting suspected etiologic agents in foods.) This verification is done in consultation with medical professionals.

Get case histories

When a complaint involves illness, complete Form C1–2, *Case History* (pages 74 and 75) either at the time of initial notification or during a personal visit or a telephone call to the person reported to be ill. Use this detailed interview approach with every person who has been identified in the initial complaint or alert, even though some may not have been ill. Continue this until sufficient information is obtained to decide whether there is, indeed, an outbreak of foodborne illness. From persons who are at risk of illness but who remained well, also get 72-h food histories and information about their activities in common with the ill persons. Information from these persons is as important to make epidemiologic associations as it is from the cases.

When it is apparent that an outbreak has occurred (see page 17 for definition of an outbreak) and a specific event has come under suspicion, substitute Form D1–2, *Case Histories Summary* (pages 76 and 77) for Form C. Form D can be used initially in many routine foodborne illness outbreak investigations where it is obvious that a common source outbreak has occurred or when all of the ill ate food together (e.g., school lunches, church supper, or banquet); at the same place (e.g., restaurants); or event (e.g., festival). This will simplify recording, because most affected persons will give similar information. At this time, notify the district, state, or provincial epidemiologist about the outbreak.

If a specific pathogen (e.g., *Salmonella, E. coli* O157:H7, hepatitis A virus) has been identified as the etiologic agent, consider developing a form for recording relevant information. Include signs and symptoms of the illness and other clinical information, the etiology of the agent, and usual methods of transmission. Computer programs (e.g., Epi Info™) can aid in the design of such forms.

Upon contact with the affected person, identify yourself and your agency and explain the purpose of the visit or call. A professional attitude, appropriate attire, friendly manner and confidence in discussing epidemiology and control of foodborne illnesses are essential for developing rapport with affected persons or their families and in projecting a good image of the investigating agency. Keep in mind that you are not interviewing someone you inspect or regulate, but that you are providing a service to the affected person. Exhibit genuine concern for persons affected and be sincere when requesting personal and confidential information. Communicate a sense of the urgency of the investigation, and emphasize that their participation will make a positive contribution for the control and prevention of foodborne illness. Parental consent must be obtained before interviewing children under 18 years of age.

After asking open-ended questions about the person's food exposures and illness history, follow up with more specific questions to fill in the details and better ensure a thorough recall. Base your level of communication on a general impression of the person being interviewed, considering information about age, occupation, education, or socioeconomic status. Tact is essential! Use either Form C or Form D, as appropriate, as a guide. State questions so

that the persons being interviewed will describe their illnesses and associated events in their own words. Try not to suggest answers by the way you phrase questions.

Fill in Form C1–2 (if appropriate) and take additional notes during the interview. Ask specific questions to clarify the patient's comments. Think questions through before conducting the interview. Realize that people are sometimes sensitive to questions about age, sex, special dietary habits, ethnic group, excreta disposal, and housing conditions. Nevertheless, any or all information of this type can be relevant. Word questions thoughtfully when discussing these characteristics and habits. Such information can often be deduced from observations. If doubt remains, confirm your guesses by asking indirect questions. Information on recent travel, gatherings, or visitors may provide a clue to common sources or events that would otherwise be difficult to pinpoint. Review known allergies, recent immunizations, recent changes in the patient's medical status, and similar information.

As persons describe their illnesses, check boxes next to appropriate symptoms or signs on Form C1. Do not ask about all symptoms or signs listed; however, ask about those marked with an asterisk if the ill person does not mention them. If there are questions, explain symptoms to the patient in understandable terms. The symptoms and signs in the first two columns of Form C1 are usually associated with poisoning or intoxication, although some occur during infections. Those in the third, fourth, and fifth columns are usually associated with enteric infections, generalized infections, and localized infections, respectively. Those in the last column are usually associated with disturbance of the central nervous system. Diseases in any category will sometimes be characterized by a few symptoms and signs listed in the other columns, and not all signs and symptoms occur for any one ailment or for all persons reporting illness. If an illness seems to fall into one of these categories, mention other symptoms in the category and record the patient's response.

Whenever possible, use physician and hospital records to verify signs and symptoms reported by patients. Clinical data may strengthen or dismiss the possibility of foodborne illness. Before contacting a physician or a hospital, become familiar with laws and codes relating to medical records to ensure that you have legal access to these records. Legal release forms may be necessary to obtain some records. Do not distribute names of patients, their other personal identities (e.g., address, phone number), or their clinical information to unauthorized persons.

Signs and symptoms will sometimes give a clue to the transmission route by indicating the organ systems affected. (See Table B, *Illnesses acquired by ingestion of contaminated foods: A condensed classification by symptoms, incubation periods, and types of agents,* pages 100–122 for listings of typical signs and symptoms and incubation periods of specific diseases.) If the early and predominant symptoms are nausea and vomiting, ask about foods or beverages ingested within the last 6 h. In these situations, suspect a bacterial (e.g., staphylococcal intoxication, *Bacillus cereus* gastroenteritis) or chemical poisoning. High-acid beverages (e.g., carbonated beverages, fruit drinks) tend to leach metallic ions from pipes and containers. If diarrhea and abdominal cramps predominate without fever, be suspicious of foods eaten 6 to 20 h

before onset of illness. Primary concerns are *Clostridium perfringens* and diarrhetic *B. cereus*. If diarrhea, chills, and fever predominate, be suspicious of foods and beverages ingested 12 to 72 h before onset of illness for salmonellosis, shigellosis, *E. coli* infections and Norwalk-like gastroenteritis.

Gather information about all foods eaten at least 72 h, and water ingested 2 wk, before onset of illness. The food, even the meal, which precipitated the illness might not be obvious; persons often have difficulty in remembering all foods eaten and water ingested during these intervals. Therefore, if the person does not remember specific foods eaten, ask about usual foods eaten and events attended during this interval. This may stimulate recall. The entries begin with the day of illness, followed by the previous 2 days. If the illness, however, began early in the day or before any of the listed meals, modify the entries on the form so that the 72-h history can be completed in the space provided on the form. If the incubation period is 3 days to a week in duration, use additional copies of form C2 and modify day or day before subtitles.

If the incubation period averages 1 to 2 wk, consider typhoid fever, cryptosporidiosis or giardiasis. Diseases with incubation periods exceeding 2 wk (e.g., hepatitis A and E) can be handled as special cases for which longer histories would be sought. In these situations, omit the 72-h food history and enter other potential vehicles (e.g., water and shellfish if hepatitis A is suspected). Enter this information in the row that cites the history of ingesting suspected food.

Other microorganisms not listed in Table B are potentially spread by food, but they are rare or not yet identified as being foodborne. These may be opportunistic pathogens, particularly for highly susceptible and immunosuppressed persons. Further investigation is needed to confirm their role in the spread of foodborne diseases.

Get information about the usual places that the ill persons eat, sources of water and ice, and places where food was consumed during the past 72 h. Ask about other risk factors for enteric illness, such as contact with young children and child care centers, animal contact, ingestion of raw foods of animal origin, and usual food preference habits. For persons who have been traveling, ask them where (both cities and rural areas) they have traveled during the incubation period of suspected agents. Consider both domestic and international travel. Determine if they ate food at unusual events or consumed foods different from their usual diet (e.g., at a festival or while on vacation). This information sometimes provides clues to common sources or to events that otherwise would be difficult to discover. Record the information on Form C.

In a protracted outbreak or when investigating an outbreak of a disease with a long incubation period, expect recall to be poor. In this situation, obtain from ill persons and others at risk a listing of their food and beverage preferences, amounts usually ingested, or their purchases of these items within the range of the incubation period of the suspected disease. As a guide, draw up a list of either foods and beverages that are commonly consumed by the affected group or foods and beverages previously identified as vehicles of the suspected disease under investigation.

Summarize data from all copies of Form C1–2 on Form D1–2 (page 76 and 77). Form D allows rapid review of all cases and controls and serves as a basis for analyzing the data.

Obtain clinical specimens

Diagnosis of most diseases can be confirmed only if etiologic agents are isolated and identified from specimens obtained from ill persons. Get specimens from as many ill persons as the laboratory can handle to confirm an etiologic agent. Also get control specimens from persons with similar exposure histories who did not become ill. People are more likely to cooperate while they are ill, and some pathogens or poisonous substances remain in the intestinal tract for only a few days after onset of illness (e.g., *C. perfringens,* Norwalk-like viruses). Other pathogens (e.g., *Salmonella, Shigella*), however, may be recovered for a few weeks. Therefore, obtain clinical specimens at the time of the initial interview during acute illness or as soon as practicable thereafter. If applicable for the disease under investigation, take specimens even after recovery because etiologic agents may remain in low populations or concentrations and changes in serologic titers can be detected. If a disease has already been diagnosed, collect specimens as listed in Table B (page 100). If a disease has not yet been diagnosed, choose specimens that are appropriate to the clinical features. Laboratory information obtained from the first patients may be useful to physicians in treating cases detected later.

In large outbreaks, obtain fecal specimens from at least ten persons who manifest illness typical of the outbreak. In smaller outbreaks, obtain specimens from as many of those ill and those at risk as practicable, but from at least two, and preferably ten, ill persons.

Before collecting specimens, review Table C, *General instructions for collecting fecal specimens* (page 123) and, if necessary, get additional instructions from laboratory personnel. Try to collect specimens before the patient takes any medication. If medication has already been taken, collect specimens anyway, and find out the kinds and amounts of medicine taken and the time that each dose was taken.

Feces. If the patient has diarrhea or is suspected of having had an enteric disease, obtain a stool specimen if practicable, or a rectal swab. Instruct patients to provide you with their own specimens by one of three means.

1. If practicable, give the patient a stool specimen container or a large disposable self-sealing plastic bag with a wooden or plastic spoon or a tongue depressor. An unused plastic bag or clean container available in the home (e.g., a jar, milk carton, or large paper cup that can be sealed) and a clean spoon or similar utensil can be used if laboratory containers are not available. Tell the patient or household member to collect the stool specimen by one of the following methods:

   a. Put sheets of plastic wrap or aluminum foil under the toilet seat and push them down slightly in the center, but not so far as to touch the water in the bowl. Sheets of paper can be tacked on the riser of a latrine and pushed down to form a depression in which to catch feces. After defecating, use a clean spoon or other utensil to transfer the amount of feces stated in Table C (page 123) into a specimen container, plastic bag or other clean container.

   b. If the stool is not liquid or explosive, float a paper towel on the surface of the water in a well-flushed toilet bowl. Defecate on the towel

and use a spoon, tongue depressor, or other utensil to collect the amount specified in Table C from the portion of the stool that remains above the water line and transfer it to the specimen container.

c. Defecate directly into the specimen container or other dry container.

2. If tests are to be conducted for bacteria and fecal swab kits have been provided, tell the patient to twist the cotton-wrapped end of the swab into the stool obtained in one of the ways described above. Follow instructions given in Table C. If necessary, use fecal-soiled toilet paper or cloth diaper and twist a swab into the top of feces.

3. If the stool is not liquid, give the patient a sterile specimen bottle containing liquid transport medium (e.g., Cary Blair, Stuart's, Amies') or a paper cup and tissues. Tell the person to put an excrement-smeared piece of tissue or toilet paper into a specimen container or plastic bag.

If you cannot deliver stool specimens to the laboratory immediately, follow instructions in Table C (page 123). Stool specimen containers for intestinal parasite examination are not suitable for bacterial or viral examinations because they ordinarily contain a preservative, such as formalin or polyvinyl alcohol. If an inappropriate transport medium is used, a specimen can be rendered unsuitable for laboratory examination.

Feces from rectal swabs. Collect rectal swabs by carefully inserting the swab approximately 1 inch (2.5 cm) beyond the anal sphincter. Gently rotate the swab. Fecal matter should be evident on the swab.

Vomitus. If the person is vomiting or subsequently does so, arrange to collect vomitus. Tell the patient to vomit directly into a sterile specimen container or a plastic bag. Otherwise, transfer some vomitus from a clean receptacle or lavatory into the container with a clean spoon. Refrigerate, but do not freeze, this specimen until it can be picked up or delivered to the laboratory.

Blood. Blood should be obtained by a clinically-trained person with a legal right to do so (check appropriate laws). Take blood if a patient has a febrile infection or when infectious agents or botulism are suspected (see Table B, pages 100–122). Collect blood during the acute phase of illness as soon as the febrile patient is seen (within a week after onset of illness) and again within 6 wk (usually 2 to 4 wk later) during the convalescent phase. If possible, collect the blood from the same patients from which stool specimens were obtained if both specimens are to be examined.

Blood is usually taken from the *medial antecubital* vein, which runs just beneath the skin of the inner elbow. Tighten a tourniquet around the upper arm to engorge the veins below, but not enough to cut off arterial circulation. Swab the anticipated puncture site with 70% alcohol and then apply 2–4% iodine and allow 1 min contact time. Insert a 3.7 cm (1.5 in.), 22-gauge sterile needle into the vein and draw 15 ml of blood (from an adult) or 3 ml (from a child) or 1–2 ml (from an infant) into a sterile syringe or evacuated sterile tube that does not contain anticoagulants. If practicable, centrifuge the blood at 1,000 rpm for 10 min; pour off the serum into small screw-cap vials and store at −18°C (0°F). If the serum cannot be separated immediately, rim the

clot with a sterile applicator stick and refrigerate at 4°C (40°F) to get maximum clot retraction if the specimen is to be stored unfrozen overnight. If centrifugation cannot be done, store the blood specimens in a refrigerator until a clot has formed, then remove the serum and transfer it with a Pasteur pipette into an empty sterile tube. Send only the serum for analysis.

Blood specimens may be tested for antibodies, pathogens, or antitoxins. If antibody studies are to be made, specimens need not be refrigerated during the day of collection unless the weather is extremely hot, but they should be kept out of direct sunlight.

Label tubes and vials at every step of serum transfer. Do not freeze whole blood because the resultant hemolysis interferes with serologic reactions. If isolation of viruses is to be attempted, however, quickly freeze tubes of clots and of serum by inserting them into an alcohol-dry ice bath. Store both serum and clot at a temperature of −70°C (−94°F) either on dry ice or in a mechanical freezer until shipped. If dry ice is used, tightly stopper the tubes and put them into a plastic bag or a metal can and fasten securely. These practices prevent a lowering of the pH of the specimens, which may be deleterious to bacteria or viruses.

Urine. Instruct patients to collect urine in the following manner. Clean the area immediately around the urethral orifice with a paper pad that has been pre-moistened with 4% tincture of iodine or other appropriate antiseptic. Then begin to urinate into a toilet and collect 30 ml (about 1 ounce) of midstream urine into a sterile bottle. Use either a second antiseptic-moistened pad or an alcohol-moistened cotton ball or tissue to clean any drops from the top or side of the bottle.

Other instructions. Follow applicable instructions given in Table C (page 123). Before or immediately after collecting clinical specimens, use waterproof permanent markers to label each container with the complaint number, case identification number, specimen number, date and time of collection, tests requested, and other appropriate information. Tightly seal all containers. Complete Form E, *Clinical specimen collection report* (page 78) for each specimen. The complaint number, case identification (ID) number, and specimen number must be entered on each report so that laboratory results can later be correlated with other data. Record on either Form C or D the type of specimen collected, and submit both the specimen and a copy of Form E to the laboratory. Send a copy of the laboratory report to the patient's physician or call if urgent.

Pick up food samples and containers that the patient collected

If the victim or other household member collected any suspected foods as instructed during initial contact, label containers with the complaint/outbreak and sample numbers. Also, collect leftovers of foods that were eaten in the last 72 h if home-prepared foods are under suspicion. Proceed as instructed in *Collect samples of suspect foods* section (page 36), and Form F, *Food sample collection report* (page 79). Record conditions of collection as called for on the forms. If the clinical data or the food histories associate the

illness with a food, caution persons not to use the remaining stocks of suspect foods until the investigation is complete. If testing a large number of samples is impractical, examine the most highly suspect food or foods first. Hold the others samples refrigerated in the laboratory for testing later, if necessary.

If the suspect food is a commercial product, obtain the original container (e.g., can, label, and lid), if feasible, even if it has been discarded. An empty container can be used to identify the processor and micro-leaks, or rinsings from these containers can be used to detect microorganisms or toxins. Check the original package or container for a code number that can be used to identify the place and time of processing. Record on Form F pertinent data such as the time and place of purchase.

Develop a Working Case Definition

Develop a working case definition to classify exposed persons as cases or non-cases. Start with the most pronounced signs and/or symptoms (such as diarrhea, vomiting) rather than nonspecific symptoms (such as nausea, abdominal cramps or malaise). For example, in an outbreak of gastroenteritis, a case might be defined as a person who was at risk and developed diarrhea within a specified period of time. Diarrhea must be defined, perhaps as three or more loose or watery stools within 24 h. An investigation might also be initiated as a result of clinical specimens yielding the same pathogen. A case definition, which is developed later in the investigation, might include either a person having signs and/or symptoms characteristic of a likely syndrome within a period of time or a person from whom a specific pathogen was isolated (See Table B).

Sometimes the first symptom or sign provides a clue to developing a case definition. Use Table B (pages 100–122) and Table D, (*Guidelines for confirmation of foodborne disease cases and outbreaks*) (pages 125–131) as guides for making case definitions. Compare each newly identified case with the definition to see whether it is part of the outbreak.

Cases may be classified into one of three categories:

- *A confirmed case* is a person who has signs and symptoms that are clinically compatible with the disease under consideration, and for which there is either (a) isolation of an etiologic agent from (or otherwise identified in) an appropriate specimen from the patient, or (b) serologic evidence of a four-fold or greater rise in convalescent antibody titer. A confirmed case must also have possible exposure to the etiologic agent within the incubation period of the disease.
- *A presumptive case* is a person who has signs and symptoms that are clinically compatible with the disease under consideration, and for which there is laboratory evidence of infection (e.g., an elevated antibody titer, but less than a four-fold increase), but the etiologic agent has not been found in specimens from patients or no specimens were collected. A presumptive case must also have possible exposure to the etiologic agent within the incubation period of the disease.
- *A suspected case* is a person who has signs and symptoms and incubation period that are clinically compatible with the disease under con-

sideration and history of possible exposure, but laboratory evidence is absent, inconclusive or incomplete.

It is not essential, however, to classify cases into these categories. Do so only if it aids in developing a final case definition or in making comparative analyses of data.

A secondary case is a person who became infected from contact with a primary (outbreak-associated) case or from a vehicle contaminated by a primary case. Onset of illness for secondary cases typically is one or more incubation periods after the outbreak-associated cases.

Consider doing analyses using case definitions of both confirmed and combined confirmed, presumptive, and highly suspect cases, and compare the results. The ultimate case definition has a tremendous impact on ability to make illness and exposure associations and to calculate probability of these associations.

Make Epidemiologic Associations

Make a preliminary evaluation of the data collected as soon as possible. If you decide that there is an outbreak, use the information that you have collected to develop an hypothesis about the causal factors.

Make time, place, and/or person associations

A time association exists if the times of onset of similar illnesses are within a few hours or days of each other. Place associations exist when persons (a) purchase foods from the same place, (b) eat at the same establishment, (c) attend the same event, or (d) reside in a place common to all. Person associations suggest a shared personal characteristic, such as being of the same age grouping, sex, ethnic group, occupation, social group, or religion. Once some of these associations become obvious, question other persons who could be at risk because of their time, place or person association with the ill persons.

Decide whether an outbreak has occurred

An outbreak is an incident in which two or more persons have the same disease, have similar clinical features, or have the same pathogen—thus meeting the case definition—and there is a time, place, or person association among these persons. A foodborne outbreak is one that is traceable to ingestion of a contaminated food. A single case of suspected botulism, mushroom poisoning, ciguatera or paralytic shellfish poisoning or other rare disease (e.g., *Vibrio vulnificus*), or a case of a disease that can be definitely related to ingestion of a food, can be considered an incident of foodborne illness and warrants further investigation.

Sometimes an outbreak of foodborne disease can be determined from an initial report simply because of the number of persons displaying certain signs and symptoms at about the same time. Many complaints, however, involve illness in only one or a few persons. It is often difficult to decide whether ingestion of a particular food and the onset of illness were associated or

coincidental. Certain diseases that are highly communicable (e.g., shigellosis and epidemic viral gastroenteritis) may result in secondary infections, person-to-person spread or subsequently contaminated food or water. Other common-source outbreaks, such as those caused by water or cross contamination from diapers in child-care centers, may also simulate foodborne outbreaks. If complaints are received from several persons who have eaten the same food or who have eaten at the same place, food is likely to be involved. Routinely reviewing the *Foodborne, waterborne, enteric illness log* (Form B, page 73) for similar complaints can often be useful in detecting time, place or person associations.

Formulate hypotheses

From time, place, or person associations that have been established or suggested by the investigation, formulate hypotheses to explain (a) the most likely type of illness, (b) the most likely vehicle involved, (c) where and the manner by which the vehicle might have become contaminated, and (d) other possible causal relationships. (The section, *Analyze data,* page 44, describes calculations that can aid in the formation of these hypotheses.) If the hypothesis relates to foodborne illness, follow the instructions given in this manual. If the hypothesis relates to waterborne illness, follow the instructions given in *Procedures to Investigate Waterborne Illness.* If the hypothesis relates to other sources, seek other appropriate guidelines.

Expand the Investigation

Test hypotheses by obtaining additional information to confirm or refute their validity. Do this by case control or cohort studies, additional laboratory investigations, and on-site investigations (e.g., hazard analysis).

Obtain assistance

If an outbreak investigation requires resources beyond your agency's capacity, request assistance from other health professionals. It is desirable to have a team including, if feasible, an epidemiologist, a microbiologist, a physician, a sanitarian, a toxicologist, and others qualified to undertake a detailed foodborne illness investigation. Such personnel can usually be provided by local, state/provincial, or national agencies concerned with health, food, environment, fisheries, or agriculture, depending on the expertise needed.

Find and interview additional cases

Continue to search for and interview ill persons who have had time, place, or person associations with the identified cases. (See the section, *Make time, place, and/or person associations,* page 17.)

Review recent complaints in the complaint log for possible outbreak-associated cases (Form B, page 73). Contact other nearby health agencies, hospital emergency rooms, and local physicians to discover other epidemiologically related cases. Call previously contacted persons to see whether they know of anyone else who has become ill or who has a common association

suggested by data in the log. Obtain reservation lists, computer records, and credit card slips from foodservice establishments to attempt to find others who were exposed.

If it becomes apparent that an outbreak is associated with a specific event or one meal, use Form D (pages 76, 77) for recording information. At this stage of the investigation, interviews can be expedited by reviewing the event or meal itself to stimulate each person's recall. Ask about specific symptoms and signs that are common to the syndrome, the time of eating the suspect meal or food, and the time of onset of illness. Mention each food served at the event or meal to which the person may have been exposed, and ask each person (whether a case or a well person at risk) which of the foods they ate.

The number of persons to be interviewed depends upon the number exposed and the proportion of them who are probably affected; if fewer than 100 persons were at risk, try to interview all of them; if several hundred are involved, interview a representative sample. Be sure to obtain clinical specimens from these cases and well persons at risk of exposure (controls). (See section on *Obtain clinical specimens,* page 13.) There may be situations where questionnaires are sent to cases and persons at risk for them to answer and return. Use either Form C or D (pages 74–77) or modified versions for this purpose. After questionnaires have been completed, summarize the data on Form D. Also, identify and interview secondary cases if they become apparent.

Find and interview controls

Statistical analyses of outbreak data cannot be performed without a group of non-ill persons (controls) or persons at risk who did not become ill. Controls are persons who are not ill but have other characteristics of the cases. If feasible, select two controls per case. A control may be a well family member or neighbor that remained well but had the same exposures. Controls also may be obtained by calling billing receipts, by contacting a neighbor, or by calling random numbers.

A common way to search by telephone for eligible controls is to add 1 to the last digit of the primary residence telephone number of the case for the first 100 telephone calls and subtract 1 from this number for the second 100 calls and so on. Call each number only once. Discontinue the call and dial the next telephone number if any of the following apply: there is no answer; the answer is by answering machine, pager or facsimile machine; the person answering is uncooperative, mentally or physically impaired, does not speak English, or discontinues the interview prematurely; answered by a person under 18 years of age; or the number is a place of business. Continue until two controls are identified for each case. Controls are also excluded if they have had diarrhea (for situations of enteric illnesses) within the past month or report illness or culture-confirmed infections of the agent under investigation of a household member.

Interview these persons to gather information essential to make statistical comparisons. This includes whether the control ate the meals or foods being statistically evaluated. Record this information on the same forms (e.g., C, D) as used for recording information from the cases.

Modify procedures, as necessary

Because no two foodborne disease outbreaks are identical, the order of the expanded investigation may not always follow the outlined sequence of procedures. Some investigative steps can usually be done simultaneously by different investigators. Although additional procedures may be required, the principles and techniques described will suffice for most investigations. Modify or develop additional forms, if necessary, to accommodate the type and amount of information that is to be collected.

SEEK SOURCES AND MODES OF CONTAMINATION AND WAYS BY WHICH THE CONTAMINANTS SURVIVED AND/OR PROLIFERATED

Conduct a hazard analysis at the site where the suspected food was or foods were produced, processed, packaged, prepared, transported, stored and/or served (i.e., where they may have been mishandled and/or mistreated), as applicable to the situation being investigated. The hazard analysis can prove or refute hypotheses developed during the epidemiological portion of the investigation. The concerns of this phase of the investigation are foods and the operations that they have undergone. Focus on source and mode of contamination and ways by which contaminants survived and/or proliferated. This phase of the investigation frequently will be at the site of final preparation of the epidemiologically implicated vehicle. However, if contamination, survival, and/or proliferation are hypothesized to have occurred before arrival at this site, initiate a traceback investigation to determine these factors (see section *Trace contamination and malpractices to their sources,* page 43).

A hazard analysis is quite different from inspections conducted during routine evaluations of foodservice, warehousing, processing and distribution facilities. If significant matters relating to food safety or quality are observed or otherwise identified during the investigation, note them and communicate them to proper authorities. Do not confuse matters of quality and aesthetics with the focuses of the investigation: contamination, survival and proliferation of infectious and toxic agents.

Plan On-Site Investigation

Identify persons responsible for operating and managing the implicated food facility before visiting the site, if feasible. Consider the types and sources of records that should be reviewed during the investigation. For example:

- Menus
- Recipes or product formulation records for foods under suspicion, with particular concern about recent changes
- Processing records (e.g., retort records, pasteurization charts)
- Operational manuals
- Hazard analysis critical control point (HACCP) monitoring records and time-temperature logs

- Previous hazard analyses and HACCP plans for products
- Process flow diagrams
- Absenteeism records
- Product testing and challenge testing results
- Records of complaints on the facility or product defects.

Use tact when requesting this information. In some states/provinces or countries some of this information is protected as a proprietary right and firms may refuse to reveal it.

Alert laboratory personnel that a field investigation will be made and get suggestions for samples and specimens that should be collected (see Tables C [page 123] and D [page 125]). Confer with them about special analyses, transport media, and sampling procedures; also make arrangements for rapid transport and testing of samples. Gather appropriate forms (E, F, G, H, I, J) and sample collection and specimen collection equipment, preferably preassembled in a kit (see *Assemble materials* section, page 4). Also, assemble thermometers or thermocouples and potentiometer, pH meter and other appropriate measuring devices that might be used during the field investigations. (See Table A [page 98] for further suggestions.)

Coordinate with the person who has regulatory responsibilities for the establishment under investigation. Invite this person to join the investigation team. Past inspection records may suggest problems, but be aware that outbreaks can occur, and often have occurred, in what have been considered good establishments and that previous records will seldom reflect the current situation. Routine inspection forms frequently do not emphasize critical operations whose failure may contribute to outbreaks. Furthermore, previous inspections may have been made months before when operations, foods being prepared, and personnel were different. Additionally, inspection may have been at a time of day when crucial preparation, mishandling, or mistreatment of foods did not occur, or the suspected food was not prepared or processed at that time.

At the establishment under investigation, plan to analyze all operations to which the foods of concern were subjected using Keys A–H. (See explanation on page 34 and the keys on pages 133–140.) Plan to stay in the establishment long enough to evaluate all suspicious processes. A full day and evening, or even longer, may be required. Do not make a routine sanitary inspection nor use the inspection form designed for that purpose. These will divert the investigation from its objectives of determining sources and modes of contamination, likelihood of microbial or toxin survival during processing, and opportunities for microbial multiplication.

Meet Managers

Introduce yourself to the person in charge and state your purpose immediately upon arrival at the place where the suspect food was processed or prepared, or where the implicated meal was served. Emphasize that the purpose of the investigation is to determine events or activities that contributed to an outbreak of foodborne disease so that preventive measures can be taken, and, hence, the episode can be controlled and will not be repeated. Attempt

to create a spirit of cooperation as workers' attitudes toward the investigative team are influenced by a positive, communicative, working relationship between management and the investigator. Consider the position, feeling, and concerns of the manager and workers; defensive reactions are to be expected. Many factors could have contributed to contamination or bacterial multiplication before foods came under the control of the manager. Assure the manager that these possibilities, when applicable, also will be investigated. Inform the manager of the proposed activities and benefits that may be gained from the findings for training workers. Get menus, recipes, information about the products prepared, product flow, names of persons responsible for particular operations, and other relevant records (see *Plan on-site investigation* section, page 20).

Take a preliminary look at the entire operation to observe conditions that may have contributed to the outbreak. At this time, if appropriate, take relevant measurements of critical operations before they are modified because of your investigation, and obtain samples of foods before they are discarded.

Maintain an unbiased attitude and answer questions—other than those concerning the identities of the persons whose common experiences implicated the food establishment. Don't be distracted, however, from the objectives of the visit, which are to determine source and mode of microbial or chemical contamination of the food; likelihood that pathogens survived any process designed to kill them or reduce their populations; and opportunities for growth of pathogenic bacteria or toxigenic molds. As pertinent information is obtained, take notes that can be used in a flow diagram on Form G, *Flow process of implicated food* (page 80), or for litigation that may be forthcoming, and record them on Form H, *Food processing/preparation history and hazard analysis report* (page 81).

Draw a Flow Diagram of Operations

Draw in pencil a separate flow chart for each food being investigated, showing each operation on a copy of Form G, *Flow process of implicated food* (page 80). Use information obtained from the manager and from recipes and preparation guidelines to start this drawing. Modify it with subsequent information obtained from food workers. This is necessary because workers do not always follow prescribed procedures, and managers are not always aware of all the activities of workers. Accompany workers on a walk through the processing steps for the suspect foods from receiving and storage to shipment or serving. Identify opportunities for contamination, survival or proliferation at each step. Compare observations made during the walk with descriptions of the process that workers and the manager gave you. Clarify and resolve any inconsistencies. In the flow diagram, each operation is represented by a rectangle, inside which is the name of the operation and other pertinent information about the operation. Arrows show direction of flow. Insert into each rectangle a symbol that represents your best estimate of: the probable type of contamination; likelihood of survival or destruction during heating or other processes designed to inactivate pathogens or toxic substances; or the likelihood of multiplication of pathogenic bacteria or toxigenic molds. Also measure or gather information about temperature and duration of the process,

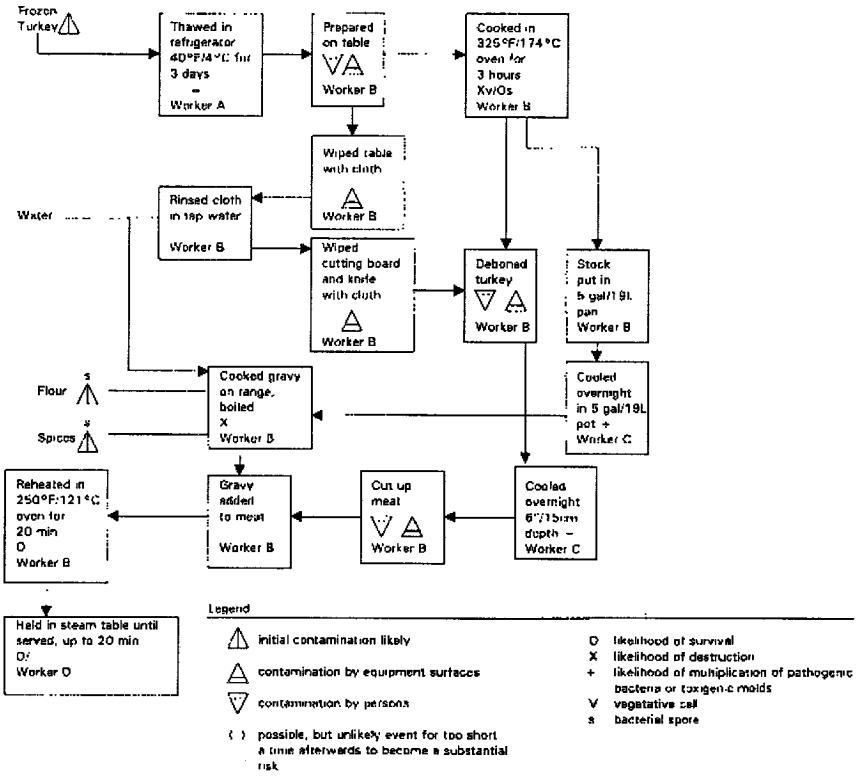

Figure 1. *Flow chart for preparation of 20-pound/9 kg turkey.*

specify size of containers and depth of food in the containers, and note name of person performing the operation on Form H (page 81). An example of a flow diagram with noted information is illustrated in Figure 1.

Interpret Figure 1 in the following manner: Raw frozen turkeys are likely to be contaminated (e.g., by *Campylobacter jejuni, C. perfringens, Salmonella, Staphylococcus aureus* and *Yersinia enterocolitica*). Subsequent contamination could have come from persons who handled the product or equipment that contacted the product while it was prepared on a table, deboned, put into pans, or cut or ground. Cross contamination could have occurred from wiping a table soiled with thaw and drip water and then using the same cloth to wipe a cutting board or knife before deboning. Cross contamination could have also resulted from Worker B, who handled the raw turkeys and later handled the cooked turkeys. Without specific temperature measurements, it is unknown whether vegetative bacteria would have survived cooking, but many types of bacterial spores would survive. The time (3 h) is short, so vegetative bacteria may have survived, particularly if the turkeys were not completely thawed. Bacterial growth could have occurred readily in the 6-inch (15 cm) depth of turkey meat and the 5-gallon (19 L) container of stock during cooling. Vegetative cells in the turkey, however, would have quite likely survived the reheating and subsequent holding in the steam table, but they would have

been killed in the gravy that was boiled. The operations of concern should relate to those specified as likely contributory factors in the keys (A–H, pages 133–140).

As information is received and recorded on the flow chart, several hypotheses will be formed. Confirm the story by talking to persons involved or who observed the operation and, if practicable, by sampling and testing foods in question and by taking measurements at critical control points during preparation at a later time. Modify the flow chart as precise information is uncovered. For certain operations that call for more detailed evaluations, checksheets may be designed.

Review Monitoring Records (if available)

Review monitoring forms for date, time and temperatures recorded, and persons doing the monitoring; entries of deviations from critical limits; notations of corrective actions taken whenever deviations were cited; and suggestions that the entries were falsified. The falsification may be characterized by the exact critical limit entered frequently, very similar daily entries, uniform entries, and illogical entries suggested by experience or knowledge of the operation and typical entries. Follow up on all suspicious entries for the dates and foods under investigation. Furthermore, observe the way workers monitor and correct deficiencies when food safety criteria are not met. Record appropriate information on Form H (page 81). Check calibration of the monitoring equipment used by establishment staff.

Interview Food Workers

Interview separately all persons (e.g., chef, processing line worker, storekeeper) who were directly involved in producing, harvesting, processing, packaging, preparing, or storing the food under investigation and other persons who could have observed these operations (e.g., plant operation supervisor, table servers, kitchen assistants, and cleaning staff). If appropriate, talk to managers and workers involved in producing, transporting, processing, packaging, preparing, or storing food at other levels of the food chain, as well as persons who prepare food at home.

Ask questions in a sequence to reveal the flow of food from time of receipt until served, taken out, delivered, or shipped. Ask each worker to describe and perhaps demonstrate the way operations were carried out. The descriptions and demonstrations may reveal whether the worker understands the food safety aspects of the job. Also, get the workers' accounts of the manner by which the food in question was prepared and treated before they had contact with the food and after it left their possession. Use all this information to revise the flow diagram (Form G, page 80).

Review recipes or formulae for ingredients that may have been the source of the contaminant (e.g., eggs for *Salmonella,* raw ground meat for *E. coli* O157:H7). Review the operational manual for procedures that may have led to contamination, survival, or proliferation of likely etiologic agents.

In foodservice establishments especially, ask preparation staff about foods that were prepared several hours or a day or more before being served at the

suspect meal, and whether there were any unusual or different circumstances or practices while the suspect foods were prepared.

Food workers who think they could be criticized or suffer punitive action because of their possible role in the outbreak do not always accurately describe or enter on monitoring forms the food preparation as it actually happened. Their descriptions and entries should be plausible and account for possible sources of contamination and suggest possibilities of survival and potential for growth of pathogenic microorganisms. If the descriptions or other data do not contain all the wanted information, reword questions and continue the inquiry. Seek confirmation of one person's story by talking to others who have knowledge of the food operation and by watching food preparation or processing practices. Be alert for inconsistencies in the accounts of different persons. Be persistent until logical accounts are obtained. Record appropriate information on Form H (page 81) and modify the flow diagram on Form G (page 80) as appropriate.

As the situations are revealed, compare them with those that are likely as shown in the keys (A–H, pages 133–140). Some of the entries specify contamination by persons who handle the foods, while others represent mistreatment of foods by persons involved with food operations.

Conduct Hazard Analysis

The investigation may involve places where foods were produced, gathered, caught, harvested, processed, stored, and/or prepared, or the means by which animals or foodstuffs were transported. Wherever possible, and perhaps at multiple places, conduct hazard analyses to determine what specific factors contributed to the outbreak. (See Keys A–H, pages 133–140.) This vital aspect of the investigation should indicate critical control points and suggest control measures and monitoring procedures to prevent a recurrence at the site and other occurrences throughout the food industry. Thoroughness in conducting hazard analyses cannot be overemphasized.

At farms, investigate (a) records or history of animal illness and visits by veterinarians, (b) changes in production practices, (c) feed sources, and (d) water quality for animal drinking, hygiene of workers, irrigation, and spraying on crops. Also investigate various operations such as (a) pesticide application, (b) fertilizing practices, (c) irrigation practices, (d) milking, (e) animal holding before slaughter, (f) fish and shellfish harvesting, (g) product washing (h) cleaning food-contact surfaces, and (i) storage procedures, as applicable. If shellfish are under consideration, evaluate records of diggers or harvesters who acquired the suspicious shellfish to identify those with a history of illegal harvesting, evidence of shellfish tag switching, or use of fraudulent tags.

At slaughter houses, watch dehairing, defeathering, washing, eviscerating, deboning, cutting up, and chilling of carcasses. At processing plants, evaluate heat processing, cooling, freezing, drying, fermenting, acidifying, smoking, packaging, storing, and/or other appropriate operations. At transport and warehouse facilities, evaluate records of food sources and consignees, opportunities for contamination based upon items previously shipped, holding temper-

atures, items stored or transported at the same time, and adequacy of refrigeration.

At foodservice establishments, retail stores, open-air markets, and homes, investigate food sources, receiving, storing, preparing, cooking, handling after cooking, hot holding, cooling, reheating, and serving foods. If applicable, investigate phases of transporting, delivery, storing and retailing. In particular, obtain information about when the suspect food was prepared, the ingredients used, and the source of any significant ingredient. Determine which workers were involved in preparing the food under investigation and the operations they performed.

Consider operations in establishments that processed or stored the food before or after the phase of the food chain that is under investigation. (See Keys A–H for listings of situations that likely contributed to foodborne disease outbreaks, pages 133–140. Evaluate whether any of them occurred.)

Complete copies of Forms G and H (pages 80, 81–83) for each suspected vehicle. The information includes sources of foods and ingredients, persons who prepared the items, procedures used, potential sources of contamination during preparation, and time-temperature conditions to which foods were exposed. Starting at the time ingredients arrived at the establishment under investigation, include all temperatures and duration of each while the food was stored, transported, prepared, cooked, heat-processed, held warm, chilled, and/or reheated, and also during the interval between serving and sampling. Pay particular attention to time-temperature recording devices and charts and associated records. Conduct tests that may reveal flow of raw fluid foods into heat-treated foods, and record this information on Form H.

Be prepared to spend time making these observations and performing tests. Based upon these discussions, observations, and measurements, revise the initial flow chart on Form G. Use the back sides or additional sheets to note additional observations or record data, as applicable.

Observe operations

Observe from start to finish operations involving the product under investigation. Initially, persons may be tense or uncomfortable when they are being watched. As time goes by or as they get busy, however, they usually process or prepare foods using their customary food processing/preparation practices because of time limitations, habits, available equipment, and established procedures that must be followed. Practices intentionally modified to impress evaluators are usually obvious and may even result in inferior products.

Determine the likelihood that incoming foods bring foodborne pathogens into the establishment under investigation. Evaluate the manner of storage to decide whether it is appropriate in relation to the food's properties and type of packaging. As raw products are processed or prepared, determine the possibility that contaminants on them are spread to workers' hands, gloves, and equipment surfaces. Follow the processing to see whether the contaminated hands or gloves touch other foods and whether other foods are processed on this equipment. Observe practices of replenishing foods on display (e.g., dumping additional foods on remaining foods). Observe the possibilities of

spreading microorganisms by cloths and sponges that are used to clean raw-food areas. Furthermore, determine procedures used for thawing frozen foods and reconstituting dried products. Watch post-heating operations to see whether the foods are likely to become contaminated by persons who handle them or by utensils or equipment. Also, determine whether any ready-to-eat foods were handled with bare hands and whether the persons handling the foods had recent illness. If so, determine the date of onset, signs and symptoms, and whether a physician was seen or specimens were collected. Observe hygienic practices, including handwashing, of persons who process or prepare foods. Observe whether quantities of potentially hazardous foods are being stored beyond the storage capacity of the facility. Evaluate the effectiveness of cleaning and sanitizing utensils and equipment by watching cleaning procedures and examining appearance of the items after cleaning. Observe all aspects of likely contributory factors that are shown in the keys (A–H, page 133–140). Modify, if necessary, the rough flow diagram and data on Form G (page 80) as a result of the observation. Record other pertinent information on Form H (pages 81–83).

Measure time/temperature exposures of foods

Check calibration of temperature-measuring devices before using them in foods, and calibrate them if necessary. Then, thoroughly clean and disinfect them. Disinfect thermocouples by inserting the sensors into a pan of boiling water for a few seconds; immersing them into a solution of at least 50 mg/L (ppm) hypochlorite for 30 sec; dipping them into 95% ethyl alcohol and immediately flaming them; or holding them over a flame from either a torch or ball of alcohol-soaked cotton. If flamed after immersing into alcohol, repeat three times. Disinfect thermometers by inserting the bayonet or bulb into boiling water or into a tube containing at least 50 mg/L (ppm) solution of hypochlorite for at least 30 sec. (In certain foodservice operations, it may be feasible to keep water boiling in a pan on a stove or range throughout the day of investigation for disinfecting thermometers or thermocouple probes.)

Measure temperatures of foods with thermocouples or thermometers. (Thermocouples measure temperature at precise locations accurately and rapidly.) Insert the sensing end of the thermocouple probe into the precise location where a measurement is wanted. (The approximate center of a food is frequently used, but under certain circumstances another internal location or the surface may be chosen.) Use bayonet-type thermocouples of appropriate length to reach the point to be measured in internal regions of food. If practicable, insert most of the shaft of the probe into the product being examined. Use thermocouples with button ends or reflecting potentiometers (temperature-measuring gun) to measure temperatures at product surfaces. If a reflecting potentiometer is used, position it close to and toward the food surface being measured. Plug thermocouple leads into a potentiometer and take readings at appropriate intervals.

If bayonet-type thermometers are used, insert the point beyond the approximate center to measure the highest temperature of a food being cooled or the lowest temperature of a food being heated. Raise and lower the thermometer as necessary to locate this zone.

Measure time with watches, timers incorporated in the potentiometer, or recording devices. Record measurements and the times temperatures were measured on Form H (pages 81–83), or make graphs from the data.

Measure product temperatures during processing and storage and record time sequences of operations. Measure ambient temperatures if they may affect product temperatures. Measure the temperature that foods of concern attained during or at the completion of initial heat processing or reheating and during post-heating temperature rise and fall until the temperature drops to 130°F (55°C). For foods cooked in retorts or pressure cookers, evaluate the operation of the retort, temperature and time of processing, venting procedure, and adequacy of sealing, rather than product temperature. Verify calibration of the establishment's time and temperature and other measuring devices.

Determine whether temperatures and holding times of foods are within a range in which bacteria can multiply, and if so, whether they are likely to multiply rapidly or slowly. Evaluate the rate at which foods cool during storage at room temperature and in refrigerators and other cooling devices. Observe and measure temperatures of foods under investigation that are stored near heat sources because they may warm to ideal bacterial incubation temperatures and possibly be maintained at these temperatures for long durations. Measure the dimensions of containers used to hold foods being cooled and depth of the food mass. Record these on Form H (pages 81–83). From these measurements, estimate probable cooling rates and the potential for bacterial growth. Determine whether covers are used (which impede cooling but may prevent further contamination or moisture and odor transfer); whether containers are stacked on top or against each other (which also impedes cooling); the location of containers in refrigerators (which may influence cooling or cross contamination); and whether there is forced-air flow or other types of rapid cooling (e.g., water/ice baths).

Plot time and temperature measurements on Form I, *Graph of time-temperature measurements* (page 84), or on graph paper. Temperature guidelines are included on Form I. They are:

- 250°F/121°C, a common retort temperature value at which bacterial spores are killed in minutes.
- 165°F/74°C, a temperature value at which vegetative forms of pathogenic bacteria are killed in a few seconds.
- 130°F/54°C, a temperature value at which vegetative forms of pathogenic bacteria are killed in a few hours.
- 120°F/49°C, a temperature value at or below which some pathogenic bacteria commence multiplication.
- 70°F/21°C, an approximate temperature value at which the bacterial log phase significantly increases and the geometric growth rate begins to slow. Rapid bacterial multiplication can occur between this value and the one listed above, particularly between 115°F/46°C (which is the optimal temperature for the growth of *C. perfringens*) and 86°F/30°C (which is the optimal temperature for the growth of *B. cereus*). Optimal temperatures for all other foodborne pathogenic bacteria also fall between these values.

- 41°F/5°C, a temperature value near that commonly recommended for the cold holding of foods.
- 32°F/0°C, a temperature value at which only a few pathogenic bacteria can still multiply over weeks of storage, though most pathogenic bacteria cease multiplication at temperatures above this value.

Label product and ambient temperature curves and give notations of operations performed and related observations at the times plotted. Interpret the data on the bases of the optimal growth temperatures for microorganisms of concern and temperature ranges at which they can multiply. Also, based on the highest temperatures reached and time-temperature exposures, interpret heating and cooling curves to determine whether the pathogens of concern could have survived heating processes, or resumed growth, if undercooked or contaminated afterward during holding or cooling. Record pertinent information on Form H (pages 81–83).

Measure pH of foods (if applicable)

Several types of pH electrodes can be used to measure the pH of foods within an establishment. Some electrodes are encased in bayonet shafts that can be inserted into foods. Others that are commonly used for testing pH of laboratory media have a flat end and can be placed on food surfaces. The conventional laboratory probe is made to test pH of liquids. If one of these is used, the food must either be liquid or ground or blended with distilled water (pH 7) that has recently been boiled (to drive off CO_2) and then cooled. Attach the electrode to a pH meter. Calibrate the meter (as recommended by the manufacturer) with at least two standard buffers (e.g., pH 4.0, 7.0 or 10.0) and compensate for temperature, if the meter does not do it automatically, before each series of tests. Measure pH of product at room temperature whenever possible. Thoroughly clean and rinse the electrode with either boiled and cooled distilled water or pH 7.0 buffer between each measurement. Record measurements on Form H (page 81).

Measure water activity of foods (if applicable)

Before each measurement, adjust the hygrometer (a_W meter) to the a_W value 0.11 or lower with a standard LiCl salt solution. To test a_W of a food, put a sample into a small plastic dish, and put it into the vapor-tight chamber of the meter. Temperature influences a_W; therefore, keep the chamber at a constant temperature. Avoid fluctuations that exceed 0.3°C. (A fan within the cabinet helps maintain uniform temperatures.) Temperature fluctuations can be minimized in the field if the sample in the chamber (with attached sensor) is kept in a styrofoam box. A temperature of 30°C is recommended because of the ease of maintaining it; if 25°C is chosen, a refrigerated system in the incubator or unit is necessary. Some instruments are designed so that the chamber is kept in a water bath or is refrigerated or heated to maintain a constant temperature.

Use a standard salt (e.g., $MgCl_2$, NaCl, KCl, K_2SO_4) or sulfuric acid (H_2SO_4) solution to calibrate hygrometers to specific a_W values according to manufacturers' instructions. (The equilibrium relative humidity [ERH] values

for certain salts at 30°C are: $MgCl_2$, 32.44+/−0.14; NaCl, 75.09+/−0.11; KCl, 83.62+/−0.25; KNO_3, 92.31+/−0.60; K_2SO_4, 97.00+/−0.40.) Select one that has an a_W value close to that of the samples to be tested. Calibrate the instrument regularly (e.g., whenever the drift exceeds 2 to 3%) to ensure a high degree of accuracy; this may require monthly calibration.

Allow time for the sample to equilibrate with respect to temperature and a_W. (This may take from a few min to several h, depending on the size of the chamber, equipment used, or the type of sample.) Read the a_W from the digital readout or the recorder plot, or determine it from a calibration curve, as appropriate for the meter being used. Run duplicate samples whenever feasible, and average them for greater accuracy. (Equilibrium is usually considered to be achieved when two consecutive hourly readings differ by less than 0.01 a_W units for direct readout equipment, or when a plateau is reached on recording equipment.) Record measurements on Form H (page 81).

Identify Contributory Factors of Outbreaks

During the investigation, identify factors that contributed to contamination and survival of the etiologic agents and perhaps also to their growth or amplification. These factors have been taken from reviews of reported foodborne disease outbreaks and outbreaks cited in scientific literature and surveillance data. They are listed and defined below according to a classification based on contamination, survival and proliferation.

Factors that introduce or otherwise permit contamination

Natural toxin. A toxic substance found in a plant or animal, or in some parts therein. For example, certain species of mushrooms contain one or more toxins.

Poisonous substance intentionally added. A poisonous substance deliberately added to a food in quantities sufficient to cause illness. Poisons added because of sabotage, mischievous acts, and attempts to cause panic or blackmail a company fall into this category.

Poisonous or physical substance accidentally or incidentally added. Although there is seldom an attempt to add poisonous chemicals to foods, these substances can reach the food from spillage or indiscriminate spraying. The misreading of labels on containers resulting in either mistaking poisonous substances for foods or incorporating them into food mixtures falls into this category. Hard or sharp objects can get into foods from (a) lack of removal (e.g., seeds, bone chips), (b) presence in soil (e.g., stones), (c) breakage during preparation (e.g., glass fragments), and (d) deterioration of equipment (metal fragments).

Addition of excessive quantities of ingredients that under these situations are toxic. An approved ingredient in a food can be accidentally added in excessive quantities so as to make the food unacceptable for consumption. Examples include too great an amount of nitrites in cured meat or excessive quantities of ginger powder in gingersnaps.

Toxic container or pipelines. The container or pipe that had held or conveyed the implicated food is made of substances that are toxic. The toxic substance either migrates into the food or leaches into solution by contact with highly acid foods or beverages. For example, a toxic metal (e.g., zinc-coated) container used to store highly acid foods, or carbonated water that backflows into copper pipes fall into this category.

Raw product or ingredient contaminated by pathogens from animal or environment. The incoming animal to be processed or the carcass or cut of meat or poultry is contaminated with pathogens when it enters the processing or preparation operations. Examples are salmonellae and campylobacters on poultry carcasses. Because these occur frequently in low populations, this contributing factor is designated only when there has been laboratory confirmation of the same marker strain of the etiologic agent in the raw food or if a traceback identifies a flock or herd as the source.

Ingestion of contaminated raw products. Contaminated products are ingested without being first subjected to any significant heat processing or cooking. Examples are raw milk and raw shellfish. This factor also includes foods that, for culinary purposes, are subjected to mild heat that is obviously insufficient to kill any pathogens present. An example is hollandaise sauce (containing raw egg yolk) that is mildly heated (i.e., heated to time-temperature exposures insufficient to kill vegetative forms of pathogenic bacteria or denature proteins).

Obtaining foods from polluted sources. Foods obtained from sources shown to be contaminated (e.g., shellfish from sewage-polluted waters; crops recently fertilized with night soil, irrigated by sewage, or sprayed with polluted water; or harvesting fallen fruit from manure-fertilized fields).

Cross contamination from raw ingredient of animal origin. Cross contamination can occur by one of several means. Raw foods or their fluids touch or drip onto foods that are not subsequently cooked. Foods not subsequently heated are processed on, or in, equipment that was previously used for raw foods of animal origin without intervening cleaning. Foods not subsequently heat processed are handled by workers who previously handled raw foods without intervening hand washing. Equipment previously used for raw foods is cleaned with cloths, sponges, or other cleaning aids that were not cleaned or sanitized; these cleaning materials are then used to wipe food-contact surfaces on equipment that will later process foods that are not subsequently heated.

Bare-hand contact by food worker. A food worker handles or otherwise touches with bare hands foods that are not subsequently cooked. This is a typical situation that precedes outbreaks caused by staphylococcal enterotoxins.

Handling by an intestinal carrier of enteric pathogens. The carrier is colonized by the pathogen and does not effectively wash hands after defecation and touches the implicated food with bare hands.

Inadequate cleaning of processing or preparation equipment or utensils. Equipment or utensils are either not cleaned between uses or the washing, rinsing, or sanitizing steps are insufficient to remove contaminants.

Storage in contaminated environment. This usually involves storage of dry foods in an environment in which contamination is likely from overhead drippage, flooding, back siphonage, aerosols or air flow, access by insects or rodents, and other situations conducive to such contamination.

Factors that allow survival of or fail to inactivate the contaminant:

Insufficient time and/or temperature during cooking or heat processing. The time/temperature exposure during initial heat processing or cooking was inadequate to kill the pathogen under investigation. This does not include inactivation of preformed heat-stable toxins. In cooking, but not retorting, it refers to the destruction of vegetative forms of bacteria, viruses, and parasites, but not bacterial spores. If the food under investigation was retorted, then spore-forming bacteria would be included.

Insufficient time and/or temperature during reheating. The time/temperature exposure during reheating or heat processing of a previously heated food (which often has been cooled, and frequently held overnight) was inadequate to kill the pathogen or inactivate heat-labile toxins. This does not include inactivation of preformed heat-stable toxins.

Inadequate acidification. Either the quantity of highly acid ingredients, the concentration of marinade, and/or the time of contact was insufficient to kill the pathogen of concern.

Insufficient thawing followed by insufficient cooking. Frozen foods were insufficiently thawed, resulting in survival of pathogens during cooking or heat processing.

Factors that allow proliferation of the etiologic agents:

Allowing foods to remain at room or warm-outdoor temperature for several hours. When foods are left at room or warm-outdoor temperature for several hours, pathogenic bacteria multiply and proliferate to populations sufficient to cause illness, or toxigenic bacteria or molds elaborate toxins.

Slow cooling. Foods are refrigerated in large quantities or stored in a manner in which cooling is impeded, allowing pathogens to multiply. Several factors may influence the slow cooling, including large masses or volumes of foods in large containers; inadequate air circulation as can occur with tight-fitting lids; stacking of pans on top of others; and crowded storage. Storing foods in large containers is the most frequent example of this type of contributory factor.

Inadequate cold-holding temperature. Refrigerators malfunction, their temperature is poorly controlled, or they have excessively high temperature settings. This is mainly a problem for cooked foods; raw foods usually spoil and will likely be discarded.

Note: The first three factors—allowing foods to remain at warm ambient temperatures, slow cooling during refrigeration, and inadequate cold-holding temperatures—can be combined into a single factor referred to as *Inadequate refrigeration.*

Preparing foods a half day or more before serving. If the interval between preparation and consumption is coupled with temperature abuse, there is ample time for pathogenic bacteria to proliferate to populations or produce toxins sufficient to overwhelm the resistance threshold of healthy adults. Shorter durations than a half day may lead to this consequence, but precise times of preparation and eating are not readily obtainable during investigations or from outbreak reports.

Prolonged cold storage for several weeks. This is a concern for psychrotrophic pathogenic bacteria (e.g., *Listeria monocytogenes,* non-protolytic *Clostridium botulinum* types B, E and F, *Y. enterocolitica, Aeromonas hydrophila*). Over sufficient durations, they multiply at ordinary refrigerator temperatures and proliferate to populations sufficient to cause illness or elaborate toxins (e.g., *C. botulinum* neurotoxins). This can be a concern also for production of histamine in certain fishes that have been stored for long durations.

Prolonged time and/or insufficient temperature during hot holding. The temperature exposure of cooked foods that are held warm is insufficient to prevent bacterial growth and may even promote such growth over time. Typically this occurs during storage in steam tables, bains-marie, thermotainers, and other hot-air cabinets.

Insufficient acidification. The concentration or quantity of highly acid ingredients, the type of acid, or the duration of marinading was insufficient to prevent multiplication of pathogens in mildly acidified foods that were not refrigerated or inadequately refrigerated. Improper mixing of acid ingredients results in insufficient acidification of the food. Therefore, the food falls into the potentially hazardous food category even though it may be suspected, because of either its name or type of usual ingredients, to have a low pH.

Insufficiently low water activity. The concentration of salts, sugars or other humectants was insufficient to prevent multiplication of pathogens in foods that have not been refrigerated or have been inadequately refrigerated. Therefore, the food falls into the potentially hazardous food category even though it may be suspected to have a low water activity by its appearance or expected process.

Inadequate thawing of frozen products. Foods are thawed in a way that is conducive to bacterial growth. The problem, however, is usually storage of foods at ambient temperatures for several hours, or at refrigerated

temperatures for several days, after the foods have thawed. When it is attempted to thaw foods in stacks of multiple units, the items on the outside frame will thaw before those in the center and reach temperatures conducive to bacterial growth while those in the interior are still frozen.

Anaerobic packaging or modified atmosphere. These factors create conditions conducive to growth of anaerobic or facultative bacteria in foods held in hermetically sealed cans or in packages in which vacuums have been pulled or gases added. All anaerobic bacteria must have a low oxygen-reduction potential to initiate growth. (This factor is restricted only to foods that are put into a sealed package or container.)

Inadequate fermentation. Starter culture failure or improper conditions to promote fermentation can result in a product (e.g., cheese, salami) containing pathogens or developing bacterial toxins.

In time, other factors will be identified. When this occurs, add them to the list. Evaluate the food and process to decide whether these events occurred. Check the appropriate boxes next to the listing of contributing factors on Form H (page 83).

Use keys to aid detection of contributory factors

Keys A–H, *Situations that likely contributed to outbreaks of foodborne diseases when*
Meat products (A)
Poultry, poultry products or eggs (B)
Milk or milk products (C)
Fish (D)
Shellfish, crustaceans or marine mammals (E)
Vegetables (F)
Fruits, nuts, spices, grains or mushrooms (G)
Formulated or mixed foods (H)
were implicated as vehicles (pages 133–140).

These provide information about many foods that have been implicated as vehicles of outbreaks and processed in various ways and for organisms likely to cause disease. Data in these tables are based on epidemiology, challenge testing, hazard analyses, and research on the ecology and toxicology of foodborne pathogens. Use the keys for guidance on likely sources and modes of contamination, likelihood of survival of heat processes, and opportunities for multiplication of microorganisms. (Each entry of these categories is indicated in the subheadings by the initials *C* for contamination, *S* for survival, and *G* for growth, multiplication or propagation). Typical vehicles are listed with subdivisions for different methods of processing. Contributory situations (which have been defined in *Identify contributory factors of outbreaks* subsection, pages 30–34) are given for raw products or ingredients before processing; processing or preparation operations that are likely to take place in food processing plants, foodservice establishments, or homes; and post-processing or preparation abuse after foods are taken home or to social events. Hence, the situations progress from the source through consumption of the foods.

See the legend at the top left corner of each key for the meaning of the symbols. The most likely events are indicated by solid squares. Half-shaded angles represent less-likely events; dots represent an even less-likely, but still possible, events. All of the situations are conditional depending upon operations being performed and other circumstances. For example, the post-processing/preparation outcomes will depend upon whether (a) the foods had been improperly processed, improperly handled, equipment improperly cleaned, (b) operations contributed to cross contamination, (c) a pathogen survived processing, (d) growth occurred after thawing, or (e) contamination occurred during or after rehydration.

Review the keys before entering the place under investigation. Once on site, evaluate situations or operations indicated by the symbols to help determine whether they contributed to the outbreak under investigation. Ensure that each of the processes having warning symbols are carefully evaluated, then documented so that the epidemiologic data base can be expanded.

Four examples demonstrate the way to use the keys. Turn to Key A *Meat or meat products* (page 133) and follow the first example.

If raw beef is suspected as being the vehicle in an outbreak of toxoplasmosis, first look down the *Process* column to *raw/heated lightly,* and then look down the *Etiologic agent* column to *Toxoplasma gondii.* Next glance horizontally across the row to the solid square (infected animal) and the half-shaded angle (cross contamination). Likely contributing factors to the outbreak are an infected animal and the carcass and cuts of meat may have become contaminated during processing.

Work through another example in the same key (A, page 133). In an outbreak of enteritis (possibly caused by *C. perfringens*) in which roast beef is the suspected vehicle, look down the *Process* column to *Cooked, pasteurized, smoked and other heat processes,* then to the *Etiologic agent* column to *C. perfringens* and read horizontally across the row for symbols that represent probable contributory factors. The source of *C. perfringens* is likely to be a colonized animal, its feces, and/or soil. It is possible, but less likely, that contamination occurred from a worker at the farm, from holding pens, or during slaughtering. This bacterium probably came with the animals to the processing plant where further spread occurred. Spores of *C. perfringens* survive cooking and will germinate, and the resulting cells multiply, if the cooked beef is improperly held hot, put into refrigerators without reducing the volume, refrigerated improperly, or kept at room temperature for several hours. (Therefore, these operations are indicated by solid blocks.) It is unlikely that multiplication occurred in the raw product. Make a thorough review of all operations indicated by solid blocks. Cross contamination, contamination by a food worker, contamination during cooling, improper cleaning of equipment, or environmental contamination at the place of processing or preparation may have occurred, but these factors are less likely than those previously stated. Thus, these operations are indicated by dots rather than squares or angles. Nevertheless, evaluate all possibilities. If the roast beef is held at insufficiently hot holding temperatures for a few hours, held at room temperature for several hours, or inadequately cooled overnight, reheating may be insufficient to kill the vegetative cells that germinated from the spores or there were post-cooking contaminants. Time-temperature abuse could occur when the roast beef

was shipped from processes or transported by caterers. Evaluate these possibilities.

A third example concerns an outbreak of salmonellosis in which chocolate candy is implicated. (See Key H, *formulated and mixed foods,* page 140.) Look down the *Food Product* column to *chocolate,* the Process column to *formulated/blended,* and the *Etiologic agent* column to *Salmonella* and glance across the horizontal row. As shown by the symbols in the row, the source of salmonellae is likely to be either an infected animal (bird, rodent, reptile) or fecal matter that directly or indirectly contaminated one of the raw ingredients (cocoa bean, coconut, nuts [see Key G] or dry milk [see Key C]). The second solid square indicates that processes, other than roasting of the bean, would not kill salmonellae. Several other possible sources of contamination are shown by the dots. Additionally, the use of water in the process or condensation in the processing plant could have possibly allowed growth on surfaces thus indicated under *improper a_w adjustments.* The *environmental contamination* mark refers to situations such as a leaking water jacket. Build-up of moisture within the package, such as results from transfer from cold to warm storage or improper packaging and storage in moist environments, could also influence microbial stability. Evaluate these possibilities. Be sure to choose keys A–G for likely contaminants of individual ingredients when investigating formulation or mixed food.

If a food has not yet come under suspicion but a disease has, the keys A–H (pages 133–140) can also be used to suggest a vehicle or possible contributory factors. For example, if *B. cereus* gastroenteritis is the disease, scan down the list in each key for this agent. It is seen under *Cooked, pasteurized, hot smoked and other heat processed meats, raw sprouts, heated beans/legumes, heated potatoes, heated vegetables, heated rice, formulated/heated gravy/soups, heated Asian mixed foods,* and *assembled/heated pasta.* Likely contributory factors are soil contamination, spore survival of heat processes, and growth during improper hot holding, improper cooling, inadequate refrigeration and room-/outdoor-temperature holding. The emetic toxin would survive reheating and the vegetative cell would survive inadequate reheating. Evaluate these possibilities.

These examples serve to show how the keys give direction for identifying the most likely factors that led to the outbreak and other possible events that influenced contamination, survival of toxins or microorganisms, or multiplication of pathogenic bacteria or toxigenic molds. Common vehicles are listed in the keys. If, however, a food under suspicion is not listed, choose a similar food and investigate the indicated operations. If a formulated food is being investigated, check likely contributory factors for each ingredient in addition to those factors listed in Key H (page 140).

Furthermore, the keys can be used to indicate critical control points of food operations and to show factors that frequently contribute to outbreaks. These can be established by glancing vertically down columns to determine those with the most entries, particularly the solid entries. Priority for preventive measures is thereby indicated.

Collect Samples of Suspect Foods

Using a menu or data from the Food-Specific Attack Rate Table (page 48) or Case-Control Exposures Table (page 50), decide which of the foods

from the implicated meal are likely vehicles and take samples of them. Check storage areas for items that may have been overlooked. Collect ingredients or raw items used in the suspect food if they are likely sources of contamination. Also, check garbage for discarded foods or containers, because suspect foods may be thrown out if it is thought that someone may have become ill as a result of eating food produced or prepared in the establishment. Interpret these results with caution, however, because post-incident contamination and/or growth may have occurred, depending upon the type of food, the ambient temperature, and the duration of the food in the container. If a food has not yet come under suspicion, collect samples of any potentially hazardous foods left from the suspect meal and of any foods available from an allegedly contaminated lot, as applicable to the situation. If deemed necessary, collect additional packages or other containers bearing the same code number so that tests can be done for microorganisms, toxins, seam defects, vacuum, leaks, or other conditions, as appropriate.

If there are no foods left from the suspect meal or lot, try to get samples of items that have been prepared subsequently to the suspect lot but in a similar manner. When particularly hazardous situations are observed, collect samples of foods before and after heating, cooling, or other operations of concern.

Collect samples of foods aseptically and put them into sterile jars or sterile plastic bags. Use utensils (e.g., knives, spoons, tongs, spatulas) that are sterile and protected by a contamination-proof wrapper until used, or wash and disinfect utensils at the time of use. If disinfecting, use one of the following procedures: (a) insert into 95% ethyl alcohol and flame three times, (b) hold over a flaming alcohol-immersed ball of cotton, or (c) expose to a flame from a torch.

Provide the laboratory with a large enough sample unit for all the necessary examinations. (A sample unit weighing approximately 200 g or measuring approximately 200 ml will usually suffice. If only one analytical test is to be done, smaller portions may be collected.) Coordinate sampling procedures with the laboratory before collecting samples.

Record temperature of the room, refrigerator, or warmer in which the food is stored just before collecting samples. Measure and record temperature of the food that remains after the sample unit has been collected. Optionally, if a plastic bag is used to hold the sample unit, squeeze the bag after filling to remove air. Wrap the filled bag around the sensing portion of the thermometer and hold it in place until the temperature stabilizes. If the sample unit is hot (>122°F/50°C), immerse it under running water or in a container of ice until the sample unit is cool to the touch.

Label the container with an establishment identification code and sequential sample unit number. Keep a log of the code numbers, date, time of sampling, type of sample and type of test to run. Complete Form F. Send a copy to the laboratory with the sample units and retain a copy for your files.

If sample units of perishable foods are not frozen at the time of collection, rapidly chill them to a temperature below 40°F/4.4°C. Keep them below this temperature until they can be examined. Do not freeze food samples because certain foodborne bacteria (such as gram-negative bacteria and vegetative forms of *C. perfringens*) die off rapidly during frozen storage. Pack sample

units with ice or another refrigerant to maintain the desired temperature during transit. Transport sample units to the laboratory in an insulated container by the most rapid means. Keep them surrounded by, but not in direct contact with, the refrigerant (to prevent freezing the sample) until either logging and preparing analytical samples or transferring to a refrigerator at the laboratory.

Choose appropriate laboratory tests

Choice of tests for the samples depends on supportive information that is wanted for the hazard analysis, type of foods being processed or prepared, and type of microorganisms or chemicals expected to be present. Aerobic mesophilic colony counts of food samples taken just after heating and again after holding give information about microbial growth during the holding period. They also can provide information on microbial inactivation (of common vegetative cells) if taken from raw materials and again after heating or from cooked foods after storage and again after reheating. These examinations have been shown to be quite useful and are within the capability of most technicians and laboratories. *Escherichia coli,* coliform, fecal coliform, and *Enterobacteriaceae* are useful indicators of post-heat processing contamination. *Salmonella* has been used to indicate survival of heat processing (e.g., egg pasteurization) or cross contamination of heat-processed foods. *Escherichia coli* can also indicate cross contamination from meat and poultry. *Staphylococcus aureus* can be used to indicate handling of heat-processed foods by human beings as well as to provide evidence of risk of foodborne diseases. Enumeration of these microorganisms and aerobic mesophilic colonies can also indicate time/temperature abuse of food.

Specific foods may be tested for the presence or quantity of certain pathogens. For example, rice and other cereals, beans, milk, and potato dishes might be tested for *B. cereus*; fish and shellfish might be enumerated for *Vibrio parahaemolyticus*; cooked meat and cooked poultry products, gravies, and beans might be enumerated for *C. perfringens.* Epidemiologic information might suggest the need to examine certain foods for particular pathogens or indicator organisms. Data in Table B (pages 100–122) and the Keys A–H (pages 133–140) can serve as guidelines on tests to run for various foods.

Recommend or Take Precautionary Control Actions (if applicable)

If there is strong evidence to form an hypothesis that the outbreak is foodborne, take applicable precautionary actions to stop the spread of the toxicant or pathogen. Avoid, however, over-interpreting data. Don't panic! The choice of action is dictated by:

- Known or suspected causal agent and the severity of its consequences
- Its source and type of vehicle involved
- Methods of food processing, packaging, and preparation to which the food has been subjected
- Distribution of the implicated food
- Availability of alternate eating sites or sources of food

- Treatment that the implicated foods are expected to receive before they are eaten
- Population at risk
- Cost of the possible actions in relationship to the risks of undesirable consequences
- Communications
- Administrative or political will to act.

Rapid and appropriate actions are obviously warranted if the disease under investigation has severe manifestations (e.g., botulism), has a high probability of extensive spread of the agent (e.g., *Shigella*), or puts highly susceptible persons (e.g., aged, infants) at risk. Take appropriate action (such as an embargo) to prevent distribution or serving of any suspect food until it is reprocessed, discarded, or proven safe. If the food is in intra- or inter-state distribution, it is appropriate to discuss findings and conclusions with epidemiologic and regulatory authorities at state/province and/or national level before taking proposed actions. Previous investigations of outbreaks give precedents for the type of action to be considered.

Verify the effectiveness of these actions by monitoring illness incidence in the population to decide whether the outbreak terminates. If the high incidence continues, consider the possibility of other transmission routes. If processed foods are shown to be the vehicle or even if they are likely to be implicated in illness, warnings to the population at risk or recalls may be initiated. Suspend the precautionary action after adequate corrections are made that can be continuously monitored, or if during the investigation it is determined that the food or establishment under investigation is not involved.

Determine Source of Contamination

A complete epidemiologic investigation of foodborne illness includes an explanation of the source of contamination as well as the manner by which the food became contaminated. The sources of etiologic agents are numerous (see Table B, pages 100–122).

Compare type of isolates from specimens with those from samples

If sources of contamination need to be identified or traced back to their source, definitive typing is usually essential. Definitive typing techniques that may be used for making associations between victim and food or between food and source of contamination are cited in Table 2.

Identify specific source of contamination

Physical substances often come from the soil from which plants are harvested; from bone, fin and claw fragments; and from damaged or worn equipment. Chemical agents usually come from substances that are added to foods or that directly or indirectly reach food during production, processing, or preparation. Microbial agents have specific sources that include soil, water, products of animal origin, and human beings who handle foods. (See Table B, pages 100–122.)

Table 2. *Examples of definitive typing schemes for foodborne pathogens*

Definitive typing scheme[1]	Pathogens
Antibiogram	*Vibrio* spp., *Aeromonas,* bacteria for which there are no other procedure
Colicin typing[2]	*Shigella sonnei*
Determination of invasiveness and enterotoxicogenesis[2]	Pathogenic *Escherichia coli, Vibrio cholerae*
Enzyme-Linked Immunosorbent Assay (ELISA)	Hepatitis A virus
M and T typing	*Streptococcus* Group A
Molecular typing[2,3]	Research applications for all major pathogens, in addition to the listed pathogens for all of the following subgroups in specialized laboratories
Plasmid profile	*Campylobacter jejuni, Escherichia coli, Salmonella, Shigella*
Polymerase Chain Reaction (PCR)	Research applications in progress for detecting most types of foodborne pathogens
Pulse Field Gel Electrophoresis (PFGE)	*Enterobacteriaceae,* enterococci, *Escherichia coli, Listeria monocytogenes, Pseudomonas* spp., *Salmonella, Staphylococcus aureus, Vibrio cholerae*
Random Amplification of Polymorphic DNA (RAPD)	*Listeria monocytogenes, Vibrio cholerae*
Restriction Fragment Length Polymorphism (RFLP)	*Listeria monocytogenes, Escherichia coli* O157:H7
Multilocus Enzyme Electrophoresis (MLEE)[2,3]	*Listeria monocytogenes, Vibrio cholerae,* research applications for other pathogens
Mouse neutralization[1]	*Clostridium botulinum* types A–E
Phage typing[1]	*Escherichia coli*; *Listeria monocytogenes*; *Salmonella typhi, typhimurium, enteritidis,* and a few other serotypes; *Shigella*; *Staphylococcus aureus*
Serotyping	*Bacillus cereus, Campylobacter jejuni, Clostridium perfringens*[4], *Escherichia coli, Listeria monocytogenes, Salmonella, Shigella, Vibrio parahaemolyticus*
Toxin identification[2]	*Bacillus cereus, Clostridium perfringens, Staphylococcus aureus* types A–E
Verotoxin detection[2]	*Escherichia coli*

[1]New commercial kits are often becoming available following research and production.
[2]Limited laboratory testing availability.
[3]Potential for use for all organisms.
[4]Previously available, and perhaps still available in some laboratories.

Raw foods. Animals may be colonized with *Salmonella, C. jejuni, Y. enterocolitica, C. perfringens, S. aureus,* and/or other pathogens. Animal carcasses can become contaminated with these pathogens during slaughtering and processing, and the meats are often contaminated by the time they arrive in kitchens (see Keys A–B, pages 133–134). If any of these agents are suspected in an outbreak, samples of meat and poultry, meat scraps, drippings on refrigerator floors, and deposits on saws or other equipment can sometimes be helpful in tracing the primary source of contamination. Swabbing surfaces of equipment (tables, cutting boards, grinders, slicing machines) that contacted the suspect food and testing the swabs for pathogens of concern can sometimes establish links in the transmission of contaminants. When this is done, however, the isolates must be definitive-typed (e.g., serotyping). This can be helpful to demonstrate the potential for cross contamination if a common utensil or piece of equipment is used for raw and then for cooked foods. Swab these surfaces with sterile swabs, moistened with a sterile solution (such as 0.1% peptone water or buffered distilled water). Break off the tip of the swab into a tube containing 5 to 10 ml of this solution or into a tube of enrichment broth for a specific pathogen. Raw ingredients, whether of animal or plant origin, may be the initial source of pathogens. Therefore, identify which ingredients were added before and which were added after any thorough cooking or heat processing. Record this information on Form H (pages 81–83).

Workers. Workers can be a source of foodborne pathogens. Enterotoxigenic strains of *S. aureus* are carried in the nasal passages of a large percentage of healthy persons. They are often found on the skin and sometimes in feces. *Clostridium perfringens* can be recovered from the feces of most healthy persons. Workers are sometimes infected with other enteric pathogens such as *Shigella, Salmonella typhi,* hepatitis A and E viruses, and small round structured viruses, which are host-adapted to human beings. (See Keys A–H, pages 133–140 and Table B, pages 100–122 for data on sources of pathogens.)

Ask food workers about their food consumption and illness history for a few days before and during the outbreak period. Evaluate whether their illness history meets the case definition. Check absenteeism by reviewing time cards and/or asking managers and supervisors. Determine the reason for any absence occurring a few days before and during the time of the outbreak. Was the absence due to a diarrheal illness? If so, compare the time of onset with the time of preparation of the epidemiologically implicated or suspect food or meal, and also with the incubation period associated with the disease under investigation. On Form C (pages 74–75), complete information about signs and symptoms, a 3-day food consumption history, and other applicable parts for each worker who reported being ill during or before the outbreak. Record this information in the *Sources of contamination* section of Form H (page 81).

Look for pimples, minor skin inflammation, boils, and infected cuts and burns on unclothed areas of the body; ask if there are any infections in covered sites. If deemed necessary, make arrangements for the workers to be examined by a physician.

If staphylococcal food poisoning is suspected, swab the lower half-inch of the nostrils of all persons who contacted the suspect food. Request a med-

ical professional to obtain a culture from any skin lesion that is found. This is done by first removing surface exudate by wiping with 70% ethyl alcohol. If the abscess is open, aspirate, if possible, or pass two swabs into the lesion and firmly sample the lesion's advancing edge. (One swab is used for culture and one for gram stain.) If the lesion is closed, aspirate the abscess wall material with needle and syringe. Tissue or fluid material is always better than a swab specimen. Sampling of surface regions can introduce colonizing bacteria that are not involved in the infection process. Rectal swabs from food workers can be useful; for example, the responsible *S. aureus* may have come from the anus or perineum. Put each specimen in an individual tube containing a sterile preservative solution or transport medium, as recommended by laboratory personnel, and send them to the laboratory.

When there is an indication that the outbreak was caused by *Salmonella, Shigella* or other organisms that cause enteric infections, collect rectal swabs from persons who handled the suspect vehicle. (Procedures are given in *Obtain clinical specimens* section, page 13.) A less desirable alternative is to give each person who handled the suspect food a suitable container for a stool specimen. Then, instruct this person in its use, and state when the specimen will be picked up or describe the procedure for sending it to the laboratory. Be aware that persons who think that they may have been responsible for an outbreak may return a specimen from someone else. Other specimens may be needed, depending on the disease that is suspected (See Table B, pages 100–122).

Complete Form E (page 78) for each specimen. Take or send specimens with the report form to the laboratory.

If the disease under suspicion is hepatitis A, shigellosis, typhoid fever, or another disease for which human beings are the usual reservoir, get information about recent illnesses of all persons who may have touched the implicated food(s).

If the same type of pathogenic microorganism is recovered from both a fecal specimen of a food worker and the suspect food, do not immediately conclude that the worker was the source. Consider the events that took place before the outbreak. A worker who ate some of the implicated food could be a victim rather than the source of the etiologic agent. A history that includes a skin infection (boil or carbuncle) or a gastrointestinal or respiratory disturbance preceding or during the preparation of the suspect food would be more incriminating.

Equipment. Evaluate the cleanliness and the manner and frequency with which equipment is cleaned. Seek opportunities for and possible routes of cross contamination between raw and cooked foods that have been processed or prepared on or by the same pieces of equipment. Collect samples from or swabs of, as applicable: air filters; drains; vacuum sweepings; food scrap piles; drawers in slicers, cutters or grinders; dried deposits on equipment; and dead ends of pipe lines. These may reflect the presence of organisms that previously were in the establishment. Chill samples, unless of dry materials, and transport them as recommended (see *Collect samples of suspect foods* section, page 36). Record information on Form H (page 81).

If injury has occurred because of biting on hard or sharp objects, examine

equipment for missing fragments or parts, chips, or other signs of damage. Observe operations with the objective of determining ways in which the objects could have been introduced into the food(s).

Trace contamination and malpractices to their sources

If field investigation failed to detect the source of contamination (e.g., infected worker or contaminated equipment) at the place of preparation, contamination could have occurred before the food or ingredient arrived at this place. Therefore, trace the implicated food backwards through distribution channels to the place of origin. This may involve interstate/interprovince and international movement of the product to processing operations, sites of harvesting, farms, herds or flocks. Each commodity has unique nuances and patterns of production and distribution. The distribution pattern may be complex because the food under investigation may have come from more than one supplier and each supplier may have distributed foods to multiple customers. Traceback investigations are done to identify distribution and sources of lots of foods that the ill persons ate.

Determine the lot involved from stock rotation practices and records, dates and quantities received, and dates and quantities used or prepared. Obtain the original shipping container or container label to determine supplier, manufacturer, grade, color, quantity, shipper, and both production and pull dates, if available and as appropriate. Also, obtain copies of invoices, bills of sale, bills of laden, air-freight bills, or receipts that document the supplier, dates of shipment and receipt, and quantities purchased. If the product requires special source labels, such as shellfish tags, obtain all tags received during the interval in question. Record information about the place of serving, place of preparation (if different), and supplier of suspect food or ingredient on Form J1, *Food Traceback Report: Places of Service and Preparation* (page 85). Send these with the report, completed forms, and copies of invoices and other shipping documents to the next level of investigation, if appropriate.

Good traceback procedures require on-site contact with firms at each level of distribution. Obtaining the necessary information by telephone only without shipping documentation may give an erroneous traceback trail.

Develop a list of shipments and their dates that could be associated with the implicated food and lots from each step in the distribution chain. Record this information on Form J2, *Food Traceback Report: Supplier to sources of implicated food/ingredient* (page 86). Include quantities and delivery times that the product was received and used on each date for each step in the distribution chain from the shipping records, stock rotation, and usage practices. Also, document outgoing quantities of products and shipping times to the next level of distribution. Use additional copies of this form as needed. Develop a timeline that is appropriate to the incubation of the disease under investigation and/or development of a parasite into the infectious stage for these shipments. Narrow the investigation to the suspected shipments most likely to have been involved. Relate these records and practices to the date of the suspected event. Be aware that more than one shipper may have been associated with the event.

Conduct hazard analyses at sites of processing, storage, harvesting, and

production, as related to the origin and transport of the suspect foods. Identify possible modes of contamination and time-temperature abuse that allowed survival and/or proliferation during shipping, handling, or storage en route; site of contamination; and any illegal activity involved (e.g., shellfish bootlegging). Record this information on Form H (pages 81–83). If deemed necessary, collect samples at the different links of the distribution chain and test them for the etiologic agent. Record this information on Form M (page 93). Look for unique and identifying markers by comparing strains of isolates of the agent from the ill persons, the implicated food at the different places of distribution and use, and the original source. (see Table 2, page 40).

The purpose of this information is to go to other establishments where the food was shipped to identify other persons at risk, whether they also became ill, and whether they ate food from the contaminated lot(s). Record information on Forms J1 and J2 (pages 85, 86). Continue to enter information about earlier distribution channels and sources as the investigation unfolds. Some suppliers implicated during the traceback will try to counter suspicion by blaming the manner of contamination on the preparer, inadequate investigation methods, politics, or inaccurate traceback procedures. Therefore, provide them with epidemiologic data (e.g., food history attack rate table, statistical analysis) and laboratory results to show the validity of the investigation, and suggest guidelines for preventive measures, to gain cooperation.

Summarize traceback information by drawing a diagram on Form J3, *Flow diagram of product source and distribution* (page 87), with time notations that depict source, dates of shipments, dates of receiving the implicated lot, and dates of preparation and storage of the implicated food. The findings provide justification for recalls, embargoes of the contaminated food, and perhaps other control or preventive measures. Take the appropriate action to prevent further illness.

The process of tracing epidemiologically implicated food back through the distribution system to the place of processing or production may involve other community, state/provincial or national agencies. Therefore, notify the appropriate agencies when traceback activity has begun and supply to them completed traceback reports and other appropriate documents to facilitate the traceback. The national agencies may provide traceback assistance, will verify traceback activities that have been conducted by state/provincial investigations, and will take over investigations when jurisdictions are exceeded. Update the flow diagram as traceback information is passed from agency to agency. Maintaining open communications between the agencies involved.

ANALYZE DATA

Organize and collate data obtained from the interviews of ill and well persons who partook of the suspect meal, who ate the suspect food, who attended a common event, or who were part of a family or group of which persons became ill. Summarize these data on Form D (pages 76, 77). Use appropriate calculations and analyses to:

- Classify the illness
- Identify affected groups

- Test the hypothesis as to whether the outbreak was associated with a common source
- Determine a vehicle
- Measure disease association
- Calculate confidence interval and statistical significance
- Determine the necessity for further field or laboratory investigation

Compare results of laboratory tests and observations made during on-site investigations and related calculations with epidemiologic data.

Plot an Epidemic Curve

An epidemic curve is a graph that depicts time distribution of onset of initial symptoms for all cases that are associated with the disease outbreak. (Each case is represented as a small square.) The unit of time used in construction of the graph depends on the interval covered by the outbreak, which will vary with the disease under investigation. For example, use a scale of days or weeks for diseases with a long incubation period, such as hepatitis A. Use a scale of hours, or groups of hours, for diseases with shorter incubation periods, such as salmonellosis. A rule of thumb is that the interval used on the x-axis should be no more than one quarter of the incubation period of the disease under investigation. Construct this graph using time of onset data from Forms C or D, employing the appropriate time scale. (Figure 2 illustrates an example of an epidemic curve.)

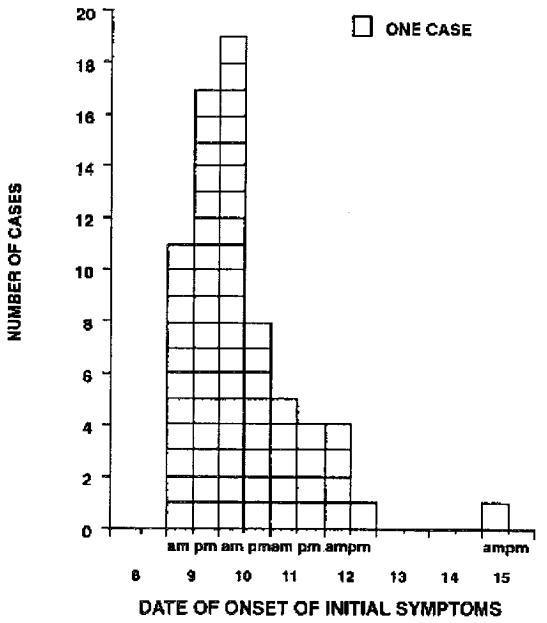

Figure 2. *Epidemic curve of a common-source outbreak.*

The epidemic curve helps to determine whether the outbreak originated from a common-source vehicle, such as water or food, or from person-to-person spread. A common-source epidemic curve is characterized by a sharp rise to a peak, followed by a fall usually being less abrupt than the rise, as shown in Figure 2. The total range of the curve is approximately equal to the duration of one incubation period of the disease. In contrast, a propagated epidemic curve is characterized by a slow, progressive rise, and the curve continues over an interval equivalent to the duration of several incubation periods of the disease.

Secondary spread can result from person-to-person contact of initial cases with previously well persons. Include these cases in the epidemic curve. Secondary spread can occur in cases of outbreaks caused by hepatitis A and E viruses, salmonellae, shigellae and *E. coli,* for example.

Determine Predominant Signs and Symptoms

A symptom is felt by a person, whereas a sign is seen by an observer. Determine the percentage of ill persons who manifest each sign or symptom (as cited in Form C or D) by dividing the number of persons reporting the given sign or symptom by the number of cases (296 in the example in Table 3, *Frequency of signs and symptoms*) and multiplying the quotient by 100. Determine which predominates by listing them in decreasing order of frequency and calculate the percentage of each as shown below in Table 3.

Table 3. *Frequency of signs and symptoms*

Signs and symptoms	Number of cases	Percent
Diarrhea	260	88
Abdominal cramps	122	41
Fever	116	39
Nausea	105	35
Headache	68	23
Muscular aches	56	19
Chills	55	19
Vomiting	42	14

Total number of cases = 296.

This information helps to determine whether the outbreak was caused by an agent that produced an intoxication, an enteric infection, or a generalized infection. Hence, it aids in classifying the illness into categories as presented in Table B. In the example given, the disease falls in the category *Lower gastrointestinal tract signs and symptoms (abdominal cramps, diarrhea) predominate.* (See Table B, pages 100–122, and Form C, page 74). Such information is useful for formulating a case definition and making or verifying a differential diagnosis. Furthermore, it aids in choosing appropriate laboratory tests.

Calculate Incubation Periods and Their Median

An incubation period is the interval between ingestion of a contaminated food (containing sufficient quantities of pathogens or concentrations of toxins to cause illness) and appearance of the first sign or symptom of the illness. Calculate the incubation period for each case. Individual incubation periods will vary because of (a) individual resistance to disease, (b) differing amounts of food ingested, (c) high or low populations of infectious agents or concentrations of toxic substances in the food, and (d) uneven distribution of the infectious agent or the toxic substance throughout the food.

The interval between the shortest and longest incubation periods is the range. Determine the median incubation period, which is the mid-point value; half the incubation periods are shorter and half are longer. Therefore, it is the mid-value of a list of individual incubation periods that are ordered from the shortest to the longest. If the series comprises an even number of values, the median is the average of the middle two values. The median, rather than the mean, is used because the median is not influenced by exceptionally short or long incubation periods that are sometimes reported in foodborne illness outbreaks, whereas the mean can be affected by aberrant values.

The median and range of the incubation periods, coupled with information regarding predominant signs and symptoms, form bases upon which to decide whether the illness in question is an infection or intoxication, and also provides subgroups for classification into categories listed in Table B (pages 100–122). This information suggests which laboratory tests should be performed.

Time of exposure may not be readily apparent because people usually eat several times each day. Therefore, the incubation period cannot always be determined for each case, and sometimes it cannot be determined for any. Careful interviewing, however, often uncovers one or more persons who ate the food in question at a specific time. Incubation periods often can be determined from such information.

Determine the Responsible (or Suspect) Meal (if applicable)

When the approximate time of exposure for groups who ate common meals is not obvious, determine it by one of four methods.

(1) Calculate attack rates among the persons who ate different meals at which the contaminated food may have been served. To do this, divide the number of persons who became ill after they ate a particular meal by the number of persons who ate the meal, and multiply the quotient by 100. Do the same for persons who did not eat the particular meal. Make the calculations for all common meals that were eaten within the duration of the incubation period of the suspect illness. The responsible or suspect meal will be the one having the highest attack rate for those who ate the meal and the lowest for those who did not eat the meal. See Table 4, *Histories of meal attendance and attack rates,* for an example. In this example, lunch on 1/17 has the highest rate for those who ate and the lowest rate for those who did not.

Table 4. *Histories of meal attendance and attack rates*

Day/ Meal	Ate/drank Ill (a)	Well (b)	Total (a+b)	Attack rate (%) (a/a+b × 100)	Did not eat/drink Ill (c)	Well (d)	Total (c+d)	Attack rate (%) (c/c+d × 100)	Difference in rates a/(a+b) − c/(c+d)	Relative risk[a] a/(a+b) / c/(c+d)	p^b value
1/16 B	52	100	152	34	58	94	152	38	−4	0.90	0.55
L	89	150	239	37	21	44	65	32	+5	1.15	0.55
D	87	150	237	37	23	44	67	34	+3	1.07	0.83
1/17 B	56	105	161	35	54	89	143	38	−3	0.92	0.67
L	106	145	251	42	4	54	58	7	+35	6.1	<.000001
D	78	130	208	38	32	64	96	33	+5	1.13	0.57

[a]See subsequent text for definitions and calculation procedures (page 53).
[b]*p* value refers to statistical significance; see subsequent text (page 56).

(2) A similar approach is to compare percent difference and odds ratios for case control studies or relative risk for attack rate studies and the probability (*p*) of chance causing the difference. The results shown in Table 4 indicate that lunch on 1/17 is highly associated with illness and that the difference in rates would occur by chance less than 1 in a million times.

(3) If the disease itself has been identified or suspected, begin at the peak of a common-source epidemic curve (page 45) and count back the average duration (hours or days) of the incubation period of the disease. The suspect meal will fall within the incubation period range and should be close to the point of median incubation period.

(4) Make a graph which lists meals and dates eaten on one axis and each case on the other. Block in the squares that have been formed whenever a patient ate a meal at the suspected place. The suspect meal will be the one that the majority or all of the patients ate.

Calculate Food-Specific Attack Rates (Retrospective Cohort Analysis) or Case-Control Exposure Percentages

Whenever a food served at a common meal or social event is suspect, prepare a food-specific attack rate table (e.g., Table 5) or a case-control ex-

Table 5. *Food-specific attack rate table*

Food/ Beverage	Ate/drank Ill (a)	Well (b)	Total (a+b)	Attack rate (%) (a/a+b × 100)	Did not eat/drink Ill (c)	Well (d)	Total (c+d)	Attack rate (%) (c/c+d × 100)	Difference in rates a/(a+b) − c/(c+d)	Relative risk[a] (a/a+b)/ (c/c+d)	p^b value
Turkey	97	36	133	73	2	23	25	8	+65	9.1	<.000001
Dressing	88	33	121	73	11	26	37	30	+43	2.5	0.000005
Peas	77	28	105	73	22	31	53	42	+31	1.8	0.0002
Rolls	50	16	66	76	49	43	92	53	+23	1.4	0.006
Pumpkin Pie	22	14	36	61	77	45	122	63	−2	1.0	0.9
Milk	12	6	18	67	87	53	140	62	+5	1.1	0.9
Coffee	59	39	98	60	40	20	60	67	−7	0.9	0.5

[a]See subsequent text for definitions and calculation procedures (page 53)
[b]*p* value refers to statistical significance; see subsequent text (page 56)

posure table (e.g., Table 6). The former is used for cohort studies when the entire group at the event is known and interviewed about illness and exposure. The case-control approach is used when all of those at risk cannot be identified or only a proportion of ill persons (cases) and well persons (controls) can be interviewed about their exposures.

Do retrospective cohort analysis (if applicable)

The food-specific attack rate table compares the illness rate among those who ingested specific foods at an event or meal to the illness rate of those who were at the event or meal but did not ingest these items. To assist in this calculation, use Form K1, *Food-specific attack rate table* (page 88) and Table 5 as guides. To calculate the food-specific attack rate for a given item, divide the number of ill persons who ingested a particular food by the total number (both ill and well) who ate the food and multiply the quotient by 100. Do the same calculations for those persons who did not eat the particular food. An example in Table 5, *Food-specific attack rates,* is:

$$\frac{97 \text{ (ill who ate turkey; a)}}{133 \text{ (total who ate turkey; a + b)}} \times 100 = 73\%.$$

Continue the calculation for each beverage or food for both those who ate the food and those who did not eat the food. Calculate the difference in rates for each food and enter the result in the column so named. Calculate a relative risk and *p*-value for all foods that have a large positive percent difference. This provides better guidance in identifying the vehicle than percent difference.

An attack rate table is useful for identifying the food that was most likely responsible for an outbreak. Identify the food in the table that has the highest attack rate for persons who ate it and the lowest attack rate for persons who did not eat it. The food with the greatest difference between these two rates is suspect. The suspicion is strengthened if this food was also ingested by the vast majority of persons affected. For example, in the above table, the attack rate for persons who ate turkey was 73%; the attack rate for persons who did not eat turkey was 8%. The difference in these two rates (percent difference) was +65%, which is greater than the difference for any of the foods listed. The relative risk shows that the attack rate for those who ate turkey was 9-times greater (73/8) than for those who did not. The data also account for all of the 99 cases, most (77) of whom ate turkey. Turkey is, therefore, the suspect vehicle although there is also a high rate difference for dressing and to a lesser extent for peas and rolls. This is probably because those who ate the turkey also ate these other foods. Negative rate differences show no association.

Some persons who did not ingest the suspect food or beverage, but who became ill, are sometimes tabulated as being ill. Plausible explanations are that some of these persons may have forgotten which food they ate; some may have become ill from other causes; a few may have exhibited psychosomatic rather than etiologic agent-induced symptoms; and if the etiologic agent was infectious in low populations, foods may have been cross contaminated. It is also not unusual for the table to include some persons who in-

gested contaminated food but did not become ill. Plausible explanations are that (a) organisms or toxins are not always evenly distributed in food and consequently some persons ingest small doses; (b) some persons eat smaller quantities than others; (c) some are more resistant to illness than others, and (d) some who became ill will not admit it.

Do case-control studies (if applicable)

If the case-control exposure table is appropriate, use Form K2, *Case-control vehicle exposure table,* (page 89) and Table 6, *Case-control exposures,* as guidelines. For both cases and controls, calculate the percentage of persons who ate a specific food and the percentage of persons who did not eat this food. Compare the two percentages and calculate an odds ratio and the *p*-value. Usually, only a portion of those ill or at risk would be chosen for comparison because not all cases and controls can be identified or interviewed. Therefore, the numbers of cases and controls would usually be lower than those in the attack rate table. The same numbers, however, are used in this example to compare the two approaches. The odds ratios and *p*-values provide higher confidence and better guidance for identifying the correct vehicle than do the percent differences, which is shown in this example.

Table 6. *Case-control exposures*

	Cases (Ill)				Controls (Well)						
Food/Beverage	Ate (a)	Did not eat (c)	Total (a+c)	Percent exposed a/(a+c) × 100	Ate (b)	Did not eat (d)	Total (a+d)	Percent exposed b/(b+d) ×100	Difference in percent a/(a+c) − b/(b+d)	Odds ratio[a] (ad/bc)	*p*[b] value
Turkey	97	2	99	98	36	23	59	61	+37	31.0	<.0000001
Dressing	88	11	99	89	33	26	59	56	+33	6.3	0.000006
Peas	77	22	99	78	28	31	59	47	+31	3.9	0.0002
Rolls	50	49	99	51	16	43	59	27	+24	2.7	0.007
Pumpkin Pie	22	77	99	22	14	45	59	24	−2	1.0	0.98
Milk	12	87	99	12	6	53	59	10	+2	1.2	0.91
Coffee	59	40	99	60	39	20	59	66	−6	0.9	0.5

[a]See subsequent text for definitions and calculation procedures (page 54).
[b]*p* value refers to statistical significance; see subsequent text (page 56).

Particular care must be taken in the selection of controls. Cases are persons who meet the case definition. Controls should not be everyone else at risk. For example, whenever laboratory-confirmed cases are used as the criterion for the case definition, persons whose symptoms are compatible with the syndromes (as listed in Table B—pages 100–122) should not be included as a case and should be excluded from the control group.

The totals for cases (ill) and controls (well) are fixed and do not change with each food unless there were unknown responses as to whether certain foods were eaten. The example in Table 6 shows a difference of 37 percent for turkey. Rolls, pie, coffee and milk can initially be excluded because the number ill does not approach the number ill for turkey. Dressing and peas need further analysis.

Table 7. *Stratified analysis comparing food-specific attack rates for eating and not eating two foods*

		Ate dressing	Did not eat dressing	Totals
Ate turkey	Ill	88	9	97
	Well	33	3	36
	Total	121	12	133
	Percent ill	73	75	73
Did not eat turkey	Ill	0	2	2
	Well	0	23	23
	Total	0	25	25
	Percent ill	0	8	8
Total	Ill	88	11	
	Well	33	26	
	Total	121	37	
	Percent ill	73	30	

Do Stratified Analysis (if applicable)

In some outbreaks, stratified analysis can be used to compare foods that have similar attack rates. As illustrated in Table 7, *Stratified analysis comparing food-specific attack rates for eating and not eating two foods* (cross table), attack rates for those eating and those not eating one food (e.g., turkey) are compared with attack rates for those eating and those not eating another food (e.g., dressing). The total values in the table correspond to values in the food-specific attack rate table, but the cells within the stratified analysis table must be obtained from data on Form D2 (page 77) or from individual food histories (Form C2, page 75).

High attack rates resulted when turkey was eaten (73% and 75%) and low attack rates when it was not eaten (0 and 8%), whether or not dressing was eaten. This was not so for dressing (73% and 0% and 75% and 8%, respectively). This comparison provides additional evidence that turkey was the vehicle in the outbreak.

Calculate Food Preference Attack Rates (if applicable)

In situations where common meals were not eaten by the ill persons, when investigating a disease that has a long incubation period (e.g., hepatitis A, typhoid fever), or when there has been a long duration between onset of illness and interviewing, a food preference attack rate table may help to identify the food vehicle. An example is shown in Table 8, *Food preference attack rate table.* In this example, a higher attack rate is observed in the group who purchased and ate Brand X cheese; a low rate occurred in the group not eating it. Thus, Brand X cheese comes under suspicion as being the vehicle of the outbreak under investigation.

Table 8. *Food preference attack rate table*

Food	Always or usually eat (Purchased within incubation period)				Never eat (Not purchased within incubation period)				Percent difference
	Ill	Well	Total	Attack rate	Ill	Well	Total	Attack rate	
Milk, Brand A	17	116	133	12.8	5	20	25	20.0	−7.2
Milk, Brand B	9	85	94	9.6	13	51	64	20.3	−10.7
Cheese, Brand X	22	102	124	17.7	0	34	34	0.0	+17.7
Cheese, Brand Y	20	125	145	13.8	2	11	13	15.4	−1.6
Cheese, Brand Z	17	100	117	14.5	5	36	41	12.2	+2.3

Make Statistical Calculations

To decide whether the observed association shows a causal relationship between exposure and disease, consider the following questions:

- How strong was the observed association between exposure and disease? Was it statistically significant?
- How consistent was the association between exposure and disease in this outbreak, and is it consistent with reports of other, similar outbreaks?
- How specific was the association between exposure and disease, i.e., did the same exposure always result in the same outcome?
- Was there a plausible time sequence, i.e., did exposure precede disease by a reasonable amount of time, considering the time of exposure and the incubation period?
- Was there a dose-response relationship? For example, were persons who consumed more food more likely to become ill?
- Is it biologically plausible that the suspected exposure caused the observed disease, so that all the data (including laboratory results from clinical specimens and food samples, epidemiologic observations, and on-site observations) fit together and make sense? There must be a rational explanation for contamination, survival, and proliferation.
- Was the same agent isolated both from persons who were ill and from the suspect food?

Measurements of association reflect the strength of the relationship between an exposure and a disease and may be thought of as the "best guess" of the true degree of association in the population under investigation. The measurement itself, however, gives no indication of its reliability, i.e., the degree of credibility to assign it.

A test of significance gives an indication of the likelihood that the observed association is due to chance. The confidence interval is a range of values within which the mean lies, with 95% probability or confidence. The chi-square test statistic is influenced by both the observed magnitude of the difference and the size of the study, but it cannot distinguish the contribution of each. The measurement of association and the test of significance (or a confidence interval) provide complementary information.

There are sources of bias that are inherent to epidemiologic data collection and testing association between disease and exposure. These include selection, information, and confound biases. An example of selection bias is when procedures used to select controls differ from those used to select cases. Cases identified often seek medical care, while less severely ill cases may not. Additionally, laboratory-confirmed cases represent only a portion of the total number of persons who became ill during an outbreak. Therefore, less severely ill cases may never become recognized. This results in an underestimate of the ill population.

Information bias may occur when retrospective questioning results in misclassification of case and control exposures due to poor recall. If misclassification is random, the result is usually an underestimate of the association; if misclassification is systematic, the association may be either under- or overestimated. Cases and controls may recall their experience differently. For example, cases who know or suspect that their illness is foodborne may recall eating a food that they did not actually eat or that they ate larger quantities than they actually did, whereas controls may not.

Confounding is caused by a second food or activity that is associated with illness and with the actual vehicle, but is not actually causative. This bias can sometimes be corrected by calculating specific rates. Biases may be compounded by the mixing of large and small effects and may even change the direction of the effect.

Anticipate biases that may exist, and try to prevent or control them and estimate the direction in which they lead. Assess the situation to avoid overinterpreting or misinterpreting the data.

Measure disease-exposure association

Two measurements of disease association—relative risk and odds ratio—are commonly used. The choice depends on the way data are analyzed. Relative risk is calculated from a cohort study, while odds ratio is calculated from a case-control study. Both calculations start with 2 × 2 contingency tables that compare ill and not-ill groups with exposure and non-exposure. An example is presented in Table 9. *2 × 2 table of data from Table 5.* Both can be interpreted as "eaters have an x-times greater risk of illness than non-eaters." Be aware of limitations of small sample sizes that result in imprecise measurements of disease and exposure.

Calculate relative risk (if applicable). The relative risk or risk ratio (RR) compares illness rates (number of ill persons/population) between populations known to have different exposure histories in a retrospective cohort study. It cannot be used in situations where the investigator chooses the proportion of ill and well persons to be interviewed. It can be used when all cases and the population exposed are known (i.e., cohort analysis). For example, it can be used for making associations of attack rates and for other situations of known populations.

Calculate the relative risks from data in Table 5, by using Form L1, *Calculation of chi square test, relative risk, and odds ratio* (page 90) and starting with data in Step 1 (see Table 9).

$$RR = \frac{\text{Illness rates among exposed group}}{\text{Illness rates among unexposed group}} = \frac{a/(a + b)}{c/(c + d)}.$$

Table 9. *2 × 2 table of data from Table 5*

	Ill	Not ill	Total
Ate turkey	a 97	b 36	a+b 133
Did not eat turkey	c 2	d 23	c+d 25

$$RR = \frac{a/(a+b)}{c/(c+d)} = \frac{97/133}{2/25} = \frac{0.73}{0.08} = 9.1$$

Interpretation:

RR = 1: No difference in risk of illness between the exposed and unexposed groups.
RR <1: Exposed group has a lower risk of illness than unexposed group.
RR >1: Exposed group has a higher risk of illness than unexposed group.

In this comparison, those who ate turkey and were ill had a much higher (approximately 9 times higher) illness rate than those who did not eat turkey. This shows a strong association between exposure and illness. It is not, however, proof of causality. This calculation assumes that other risk factors for those who ate turkey (exposed) and those who did not eat turkey (non-exposed) are approximately equal.

Calculate odds ratio (if applicable). The odds ratio (OR) is used in situation where it is not possible to obtain illness data on everyone who was exposed to a potential hazard. In such studies, the exposure histories of people with the illness (cases) are compared to the exposure histories of "similar" people (e.g., similar age, live in the same or similar neighborhood, attended the same event, and perhaps have other attributes in common) who did not become ill (controls). It is impossible to calculate true risk from a case-control study, but the odds ratio is used as an estimate of the risk.

$$OR = \frac{\text{Odds of exposure among cases}}{\text{Odds of exposure among controls}} = \frac{a/c}{b/d} = \frac{ad}{bc}.$$

Using the 2 × 2 contingency table (see Table 9, page 53 and Form L1, page 90), fill in cells a, b, c, and d. Use data for turkey in Table 6 (page 50) to calculate the odds that the people who became ill were more likely to have eaten the food in question than the people who did not become ill, as shown in Table 9. (Although the data comes from Table 6 rather than Table 5, the same numbers and totals, however, apply.)

$$OR = \frac{ad}{bc} = \frac{97 \times 23}{36 \times 2} = \frac{2231}{72} = 30.9$$

Interpretation:

OR = 1: No difference in exposure between cases and controls, therefore the exposure being examined was not associated with the illness.

OR < 1: The cases were less likely than controls to have been exposed to the suspect agent.

OR > 1: The cases were more likely to have been exposed to the suspect agent. Therefore, exposure may have contributed to illness, which is the situation in the example of data from Tables 6 and 9 (pages 50 and 54).

In this example, the odds of exposure were much greater for the cases than for the controls. Therefore, the odds of exposure (eating turkey) were greater for the group that was ill than for the group that was not ill, and turkey is likely to be the vehicle of the etiologic agent.

Another example is given in Table 10. In this situation, five cases of salmonellosis were reported and interviewed; three had eaten ice cream. Ten healthy persons were identified as controls; they had eaten at the same place.

Table 10. *Outbreak table*

Eating history	Case	Control	Total
Ate ice cream	3	3	6
Did not eat ice cream	2	7	9
Total	5	10	15

$$OR = \frac{ad}{bc} = \frac{3 \times 7}{3 \times 2} = \frac{21}{6} = 3.5$$

In this example, the OR is greater than 1; this means that the ill group was more likely to have been exposed to the suspect agent than the control group. Therefore, exposure may have contributed to illness, but the numbers used for comparison are quite small and subject to error.

Evaluate confidence intervals for measurements of association

Both relative risk (RR) and odds ratio (OR) are point estimates of the true degree of association between exposure and disease. For each of these estimates, it is possible to calculate the plausible range of values for which there is a 95% chance that the range includes the "true" RR or OR. In other words, there is only a 5% probability that such results occurred by chance. The size of this confidence interval gives an indication of the precision of the point estimate, which is influenced by the sample size. If the confidence interval encompasses the value 1.0, that measurement of association (RR or OR) is not statistically significant; the observed association between illness and exposure could be due to chance.

Confidence intervals (CI) can be calculated by statistical computer packages (e.g., Epi Info™). For the previous examples, the 95% confidence intervals are:

Table 4:	RR = 6.1	95% CI = 2.4–15.9
Table 5/9:	RR = 9.1	95% CI = 2.4–34.6
Table 6/9:	OR = 31	95% CI = 6.9–278
Table 10:	OR = 3.5	95% CI = 0.23–59.1 (encompasses 1)

Interpretation: The 95% CI around the two risk ratios is much smaller than that around the odds ratios because the former is based on larger samples. In Tables 6 and 9 one cell is small, which influences the range of the 95% CI around the relative risk value. The odds ratio of 3.5 is not statistically significant because the 95% CI includes the value of 1.0 and the CI has quite a large range, which is related to the small numbers.

Test statistical significance

Statistical significance tests calculate the probability (p) that the differences in illness rates between those who ate the suspect food and those who did not eat this food were due to chance. Test relative risk or odds ratio values that exceed 1.0 to determine their statistical significance. Such testing can provide a degree of confidence that a particular exposure is the vehicle even without laboratory confirmation. Two tests, chi-square (X^2) and Fisher's exact, are commonly used for this purpose. Computer programs (e.g., Epi Info™) are available for making such calculations, but a review of procedures for performing these calculations is given for those who do not have access to computers or appropriate software.

Initially a null hypothesis (H_0)—a hypothesis worded in a negative way—is stated. For example, a statement might be, "There is no difference in attack rates for those who ate turkey and for those who did not eat turkey." Or, "There is no association between eating turkey and getting ill." After statistical significance tests have been done, decide whether to accept or reject the null hypothesis.

Calculate chi-square (X^2) (if applicable). The chi-square (X^2) test provides a means of comparing an observed illness distribution with an expected distribution. Observed distribution is the data used to calculate incidence rates such as food-specific attack rate. Enter case-control vehicle exposure percentages data on Forms L1 and L2 (pages 90, 91), as applicable. Then, calculate the expected distribution. The expected distribution refers to a situation in which the attack rates would be expected to be the same among those who ate the suspect food and those who did not (indicating that illness was unrelated to food exposure). The level of confidence associated with obtaining different attack rates purely by chance (that is, not attributable to any cause) must be chosen by the investigator. The most common confidence level used is that of 95%, which implies that chance alone would account for 5 out of 100 (1 in 20) sets of attack rates being statistically different.

Next, calculate the X^2 statistic and determine the probability (p value), following the step-by-step procedure outlined in Form L1, (page 90). Take

the example of turkey from Table 5 or 6. Fill in cells, a,b,c and d from that table. Then fill in the marginal totals, a+b and c+d, from the table and record under substeps *i* and *ii*. Add a+c and b+d and fill in the other marginal tables and substeps *iii* and *iv*. Add the marginal totals (a+b+c+d) to obtain *n* (substep *v*). Therefore, the observed (outbreak) table would be as shown in Table 9 (page 54).

Do calculations to complete the expected table (step 2, Form L1). To do this:

vi. Multiply *i* × *iii* (133 × 99) and then divide the product by *v* or 158 to get a_e = 83.3; round off quotient to the nearest whole number (83) and enter result into the a_e cell.

vii. Subtract a_e (*vi*) (83) from *i* (133) to get b_e = 50 and enter remainder into the b_e cell.

viii. Subtract a_e (*vi*) (83) from *iii* (99) to get c_e = 16 and enter remainder into the c_e cell.

ix. Subtract c_e (*viii*) (16) from *ii* (25) to get d_e = 9 and enter remainder into the d_e cell.

The expected table would be as shown in Table 11.

Table 11. *Expected table calculated from data in Tables 5 and 6*

Eating/drinking history	Ill/Case	Well/Control	Total
Ate turkey	83 (a_e)	50 (b_e)	133 (a+b)
Did not eat turkey	16 (c_e)	9 (d_e)	25 (c+d)
Total	99 (a+c)	59 (b+d)	158 (n)

The chi-square test is only accurate if all cells of the *expected table* are 5 or greater. Note that one cell in the *observed (outbreak) table* was 2 (which is less than 5), but all of the cells in the calculated expected table are 5 or greater. Therefore, the X^2 test can be used to test the difference between the outbreak table and the expected table. (If any or all cells in the expected table are less than 5, skip steps 3 and 4 on Form L1 and proceed to step 5 on Form L2, page 91). Proceed with the X^2 calculation as indicated on Form L1 (page 90).

x. Multiply a × d (97 × 23 = 2231) and enter product on Form L1.

xi. Multiply b × c (36 × 2 = 72) and enter product.

xii. Subtract *xi* from *x* (2231 − 72 = 2159) and enter the remainder; note that if *xi* is <0 the negative sign is dropped.

xiii. Divide n by 2 (158 ÷ 2 = 79), and enter quotient rounded to a whole number.

xiv. Subtract *xiii* from *xii* (2159 − 79 = 2080) and enter remainder.

xv. Square *xiv* (2080^2 = 4,326,400) and enter product.

xvi. Multiply *xv* by n (4,326,400 × 158 = 683,571,200) and enter product.

xvii. Multiply *i* (a+b) × *ii* (c+d) × *iii* (a+c) × *iv* (b+d) (133 × 25 × 99 × 59 = 19,421,325) and enter product.

xviii. Divide *xvi* by *xvii* (683,571,200 ÷ 19,421,325 = 35.2) and enter quotient, rounded to one decimal place. This is the X^2 value.

xix. Convert X^2 (35.2) to probability (see table in step 4, Form L1). Because 35.2 is greater than 7.88, the probability (*p*-value) is <0.005, which is the smallest *p* value listed in this particular table.

The calculated X^2 value of 35.2 has a probability (*p*-value) of much less than 0.005. This is highly significant, as it indicates that the probability of the observed difference in the attack rates occurring by chance alone is much less than one time in 200 (or <5 times in 1,000). (In fact it's less than 1 in 10,000,000.) Therefore, it is highly probable that something other than chance was responsible for the difference between the observed attack rate and the expected attack rate. For instance, the turkey could have been contaminated by large populations of pathogens or a toxin and as a result could be the vehicle in the outbreak. Compare the results of the relative risk or odds ratio calculation with the X^2 calculation of turkey data from Tables 5 or 6. The X^2 calculation and associated *p*-value confirms that the relative risk and odds ratio are statistically significant.

Calculate Fisher's exact probability (if applicable). The Fisher's exact test calculates the *p*-value directly. It is particularly useful for calculations involving small numbers. There are two different ways to test the null hypothesis by the Fisher's exact test: a one-tailed test and a two-tailed test. The one-tailed test assumes a directional null hypothesis. The two-tailed test assumes a non-directional null hypothesis, as in the X^2 test. (Both are calculated by some statistical programs, e.g., Epi Info™.)

In any situation in which one or more of the cell frequencies in the expected table is less than 5, or where any of the marginal totals in the outbreak table are less than 10, Fisher's exact test should replace the chi-square test. Fisher's exact test may be used to analyze any outbreak table. However, if the cell values of the outbreak table are all fairly large (i.e., >5) then the number of calculations required to determine the *p*-value for Fisher's exact test becomes large. A high speed computer relieves this problem. Factorials are used in this calculation. [A factorial (!) is a number multiplied by all possible lesser whole numbers. For example, $5! = 5 \times 4 \times 3 \times 2 \times 1 = 120$. Note that $0! = 1$.]

An example of a one-tailed null hypothesis is, "The attack rate for those who ate ice cream is not higher than the attack rate for those who did not eat the ice cream". To calculate the one-tailed Fisher's exact test, the same 2×2 table (see Form L2, *Calculation of Fisher's exact test,* page 91) is used for Fisher's exact calculation as is used for X^2 calculations (see Form L1, page 90). The outbreak table (data in Table 10, concerning cases alleged to be associated with eating ice cream) is given again, but modified by the inclusion of attack rates for the example (Table 12, *Outbreak table for case-control study associated with consumption of ice cream*). Calculate *p*1 from data in the 2×2 table. The *p*-value for the observed distribution must be added to *p*-values of those distributions that are more extreme in the same direction. Thus, develop all possible 2×2 tables that represent a more extreme result (i.e., greater difference in attack rate) than that of the outbreak

table, but maintain the same marginal totals. This is done for $p1.2$ by decreasing the value in the c cell by one (i.e., $c - 1$). $p1.3$ is the p-value for the table created when $c - 2$ is substituted for c in the outbreak table. Continue for $p1.4$, if necessary, and through $p1.x$ until either a,b,c or $d = 0$. When making manual calculations or using a hand-held calculator, all possible cancellations must be done, first for the factorials and then for the numbers resulting from converting the factorials to whole numbers before starting calculations. Tables 13 and 14 illustrate more extreme results. Observe that the totals (a+b, c+d, a+c, b+d, n) remain fixed while the "c" cell value is decreased by one. Each different cell frequency will have an associated 2×2 table, which will be more extreme than the previous one. If any of the cell frequencies is zero, a more extreme 2×2 table is not possible. The p-value for the test become $pn = p1.1 + p1.2 + p1.3 + \ldots + p1.x$, where $p1.1$ is the p-value associated with the outbreak 2×2 table. This is necessary because the true p-value is the probability associated with getting an attack rate difference of at least as large as that encountered in the outbreak table.

Table 12. *Outbreak table for case-control study associated with consumption of ice cream*

Food consumption history	Case	Control	Total	(Attack rate)
Ate ice cream	3	3	6 (i_1)	(50%)
Did not eat ice cream	2	7	9 (ii_1)	(22%)
Total	5 (iii_1)	10 (iv_1)	15 (v_1)	(33%)

vi. To continue the example (see calculation approach in Form L2, step *vi–x*):

$$p1.1 = \frac{6!9!5!10!}{15!3!3!2!7!}.$$

vii. To simplify these mathematics, cancel factorial values

e.g., $\frac{5!}{3!} = \frac{5 \times 4}{1}$. Therefore, $p1 = \frac{6 \times 5 \times 4 \times 9 \times 8 \times 5 \times 4}{15 \times 14 \times 13 \times 12 \times 11 \times 2}$.

viii. To further simplify these mathematics, cancel whole numbers. Therefore,

$$p1.1 = \frac{3 \times 4 \times 5 \times 4}{7 \times 13 \times 11} = \frac{240}{1001} = 0.240.$$

(Note: This p-value (0.24) shows that, in 24 out of 100 times, chance could be responsible for the results; therefore, no further calculations need to be done for these data. The calculation, however, is carried on to show the procedures used.)

Table 13. *A more extreme table*

Food consumption history	Case	Control	Total	(Attack rate)
Ate ice cream	4	2	6 (i_2)	(67%)
Did not eat ice cream	1	8	9 (ii_2)	(11%)
Total	5 (iii_2)	10 (iv_2)	15(v_2)	(33%)

Table 14. *A more extreme table yet*

Food consumption history	Case	Control	Total	(Attack rate)
Ate ice cream	5	1	6 (i_3)	(83%)
Did not eat ice cream	0	9	9 (ii_3)	(0%)
Total	5 (iii_3)	10 (iv_3)	15 (v_3)	(33%)

$$p1.2 = \frac{6!9!5!10!}{15!4!2!1!8!} = \frac{6 \times 5 \times 4 \times 3 \times 9 \times 5}{15 \times 14 \times 13 \times 12 \times 11} = \frac{45}{1001} = 0.045$$

$$p1.3 = \frac{6!9!5!10!}{15!5!1!0!9!} = \frac{6 \times 5 \times 4 \times 3 \times 2}{15 \times 14 \times 13 \times 12 \times 11} = \frac{2}{1001} = 0.002.$$

x. Calculate p (pn) value for the one-tailed test

$$p = p1.1 + p1.2 + p1.3 = 0.240 + 0.045 + 0.002 = 0.287.$$

The p value from the above example for the one-tailed Fisher's exact test is 0.287, which is rounded off to 29 times in 100 that such results would occur by chance alone. A p value of less than 0.05 (5 times in 100 by chance alone) is necessary to consider the difference significant. In this example, although the cases were more likely to have eaten ice cream than the controls (as indicated by the odds ratio) this difference was not statistically significant. This result confirms the earlier finding that the 95% confidence interval of the odds ratio included the value 1.0, and thus the odds ratio was not statistically significant (see page 54).

In the two-tailed test, no assumption is made regarding which group (those who ate or did not eat ice cream) would have the higher attack rate. Therefore, the difference could be in either direction. For a two-tailed table, set up the same sort of tables and calculate p-values for the worst case in the opposite direction as done in the one-tailed test. The a+b (or i) is where the maximal number of the unexposed persons and the minimal number of the exposed persons were ill. (Therefore, the number in the 2 × 2 table's "a" cell may be, but is not necessarily, 0.) Adjust the values in all other cells, but maintain the same marginal totals. Continue increasing the number in the "a" cell by one in subsequent 2 × 2 tables and adjust the other values in the other cells. Do this until the difference in percents becomes less than the attack rate for the totals (33% in the example). Examples are shown in Tables 15 and 16. (Note: The one-tailed calculation for the p-value, 0.24–0.29, shows that chance could be responsible for the results, and, therefore, no further calcu-

lations need to be made for this data. However, the calculation for the two-tailed test is carried on to show the procedures that would be used if the calculated p-value was less than 0.05.)

Table 15. *Table for data if no one was ill*

Food consumption history	Case	Control	Total	(Attack rate)
Ate ice cream	0	6	6 (i_3)	(0%)
Did not eat ice cream	5	4	9 (ii_3)	(56%)
(percent difference)				(−56%)
Total	5 (iii_3)	10 (iv_3)	15(v_3)	(33%)

Table 16. *Table for data if one person was ill*

Food consumption history	Case	Control	Total	(Attack rate)
Ate ice cream	1	5	6 (i_3)	(17%)
Did not eat ice cream	4	5	9 (ii_3)	(44%)
(percent difference)				(−27%)
Total	5 (iii_3)	10 (iv_3)	15(v_3)	(33%)

Because the percent difference in Table 16 and all such tables with increasing number of ill persons is less than that of the totals, there is neither the need to go further nor to use this table in the calculation. Therefore, data from Table 15 is used to make calculations as above for $p1.4$.

$$p1.4 = \frac{6!9!5!10!}{15!0!6!5!4!} = \frac{9 \times 8 \times 7 \times 6 \times 5}{15 \times 14 \times 13 \times 12 \times 11} = \frac{6}{143} = 0.042.$$

Add this p-value (or additional p-values if data from multiple 2 × 2 tables are used) to the total for the one-tailed Fisher's exact test to get the p-value for a two tailed Fisher's exact test.

xi. Calculate p (pn) value for the two tailed test = p-value for one-tailed test + $p1.4$

$$p1 = 0.287 + 0.042\ (p1.4) = 0.329$$

The p value from the above example for the two-tailed Fisher's exact test is 0.329 (meaning that 33 times [rounded off] in 100 such results would occur by chance alone). No association is observed because a p value of less than 0.05 (5 times in 100) is necessary to consider the difference significant. Therefore, as stated above for interpretation of the one-tailed test, the attack rate for those who ate ice cream is not higher than the attack for those who did not eat the ice cream. This result confirms the earlier finding that the 95% confidence interval of the odds ratio included the value 1.0, and thus the odds ratio was not statistically significant.

These calculations of probability are based on a small number of people.

If additional cases and controls could be interviewed, the difference in exposure may become more pronounced and possibly statistically significant; however, additional cases and controls also could provide more evidence that there is no statistical significance.

Interpret Results and Test Hypotheses

Record all laboratory results (e.g., specimens from cases, food samples, specimens from workers, environmental samples) on Form M, *Laboratory results summary* (page 93). Compare these results with epidemiological data and on-site observations. Use data obtained throughout the investigation to test hypotheses formulated during the investigation. Each of the following factors should be consistent with the suspected agent:

- incubation period
- type of illness
- duration of illness
- population affected
- contributory factors leading to contamination of the food, survival of the pathogens from the effects of the process, and proliferation or concentration of the etiologic agent.

Use applicable statistical tests to evaluate the significance of data generated during the investigation, but be aware of associated biases.

The agent responsible for the outbreak can be determined by:

- isolating and identifying pathogenic microorganisms from patients
- identifying the same strain of pathogen in specimens from several patients
- finding toxic substances, or substances indicative of pathological responses, in specimens
- demonstrating increased antibody titer in sera from patients whose clinical features are consistent with those produced by the agent.

The presence of some pathogens (e.g., *Salmonella, Shigella, E. coli*) in the epidemiologically implicated food is sufficient for confirmation. For other pathogens (e.g., *S. aureus, C. perfringens*), however, large numbers (e.g., 100,000/g or ml) must be recovered from foods. (See Table D, page 125). The large numbers indicate proliferation after contamination.

Sometimes laboratories test foods for indicator organisms (e.g., aerobic mesophilic colony count [standard plant count], coliform, fecal or thermotolerant coliforms, *Enterobacteriaceae,* staphylococci) rather than pathogens. The finding of these bacteria, even in high populations, in a food does not indicate that the food was a vehicle. High aerobic colony counts indicate that one of two situations occurred. The first is that the raw food or ingredients contained high populations of microorganisms, and that the product received no or insufficient heat or other potentially lethal treatment to sufficiently decrease the population. The second is that the food was held at temperatures conducive to bacterial growth long enough to allow surviving spores to germinate and the resulting cells to multiply, or long enough to allow multiplication of contaminants that reached the product after a lethal process. Path-

ogens, if present, may or may not have multiplied along with the other flora; competitive flora may inhibit the growth of pathogens. The presence of soilborne bacteria would be expected in foods grown in soil or exposed to it during growing and harvesting. Similarly, marine bacteria would be expected on raw seafoods. Fecal-associated bacteria (e.g., coliforms, fecal/thermotolerant coliforms and *Enterobacteriaceae*) are likely to be found on raw foods of animal origin. Their presence in heated foods, however, suggests post-processing contamination. Large populations of them suggest that they multiplied afterwards. Staphylococci on cooked foods indicate post-cooking handling.

To confirm involvement of a suspect food, the same organism, toxin, or chemical markers must be found in the epidemiologically implicated food as were found in specimens from patients. The organism may be identified by serotype, phage type, immunoblotting, plasmid analysis, antibiotic resistance patterns, restriction endonuclease analysis, or nucleotide sequence analysis. (See Table 2, page 40.) Even when clinical specimens are not available, a vehicle can be identified, at least circumstantially, by detecting toxic substances (such as zinc or botulinal toxin), by isolating a significant number of specific pathogens (such as 100,000/g or more *S. aureus* or *C. perfringens*) from the food, or by recovering enteric pathogens (such as *Salmonella*) from a food by enrichment techniques. The food from which these findings are made also should be epidemiologically suspect as a result of analysis of the food-specific attack rate table or case-control study, and the symptoms reported by the ill should be consistent with those produced by the agent that has been isolated from the implicated food. (See Table E, *Guidelines for confirmation of vehicle responsible for foodborne illness,* page 132).

Remember that testing samples can never replace observations, but test results can provide supportive data and often prove hypotheses. Take caution, however, when interpreting results of laboratory analyses. Negative results do not mean that pathogens are absent. Detection of the etiologic agent is more probable when the individual items or lots of food are heavily contaminated. Hence, a greater number of samples will be required for detection when the level of contamination is low. Counts follow probability distributions, which appear as a bell-shaped curve with the apex at the median, and therefore individual counts may have a considerable range.

Contamination of an individual food item is seldom homogeneous. To be homogeneous, solid foods must be ground or otherwise mixed thoroughly and liquids must be mixed. Only some portions of a particular food may be touched by a contaminated bare hand, or only some portions may contact a contaminated equipment surface. In time, the contamination is wiped, diluted, or removed from the contaminated surface. Therefore, the contaminants progressively diminish as the multiple items or surfaces of the same item continue to contact other surfaces. Many foods are solid, and spread of the contaminants throughout them is unlikely.

Furthermore, all items are not stored under identical circumstances, so propagation of bacteria may be likely in one or some items but not others, depending upon storage conditions. Also, in any one item, multiplication of bacteria varies in different regions of the food. For example, bacterial growth may occur near the geometric center but not near the container surfaces of

an item stored in a refrigerator because heat dissipates more rapidly at the surfaces exposed to cold air or surfaces. Growth of normal contaminating bacteria may overwhelm the pathogen, making its isolation difficult. Between the time the implicated food was eaten and the time samples were collected, pathogens may grow during storage to populations different from those ingested. Counts from such samples are difficult to interpret.

During exposures to heat or other processes that are detrimental to etiologic agents, time/temperature exposures vary in different portions of a food item. Therefore, survival of etiologic agents in the different regions of a food varies. Keep this information in mind when interpreting the laboratory results of a sample.

Unfortunately, the specific etiologic agent cannot be identified in many foodborne disease outbreaks. This is because food samples and clinical specimens have not been collected at an appropriate time, have been held too long, are too small a volume for effective analysis, or have not been examined for the appropriate agent.

The history of the way the food was produced, processed, prepared, or stored must reveal opportunities for contamination and, where applicable, for survival and growth of pathogens; otherwise, the history as recorded is incomplete or in error. If necessary, question food workers again and seek additional information, or look for inconsistencies in their stories that may suggest where contamination or other mishandling of the food occurred. The source of the causative agent can often be traced by recovering the agent from raw foods, food ingredients, equipment, food workers, or live animals or their environment. Definitive typing of isolates is required for confirmation (See Table 2, page 40.) Such findings must also be supported by a history that would preclude the possibility of contamination from another source.

In practice, few investigations ever document and confirm all factors from the source of contamination to the onset of illness. Seldom is this even possible. Nevertheless, strive for the most complete investigation possible.

MAKE RECOMMENDATIONS FOR CONTROL

After the hazard analysis has been completed, or as hypotheses are proved, take control actions or make recommendations to prevent further spread of the etiologic agent. These actions should be implemented in the place that the epidemiologically implicated food was eaten or purchased and other places to which a traceback investigation led. Record the action taken or recommended on Form N, *Control actions taken and preventive measures recommended* (page 94). The following actions may be considered for the foods, personnel, or establishment involved.

Exclude infected persons from handling foods. Infected food workers are usually excluded from work when they show signs or describe symptoms of illness. Consecutive microbiological tests over an interval of several days or weeks may be necessary to demonstrate clearance of a pathogen, but despite this the pathogens may not be recovered during the examinations. Nevertheless, workers usually can return to work after recovery without such testing, if they practice good personal hygiene and are ad-

equately supervised. If the illness appears to be caused by an infectious agent and there is no test for that agent (e.g., Norwalk-like viruses), take the following actions: (a) intensify hand washing practices and (b) implement a no bare hand-contact policy for ready-to-eat foods. There are many sources of contamination other than workers, and workers who appear to be well may be infected without signs and may have practices as poor as or worse than those of the excluded worker(s).

Seize, detain (embargo), stop distribution, remove, recall, reject or destroy the epidemiologically implicated lot. Take appropriate action—depending upon the type and degree of contamination and the estimated extent of the contamination—to stop the outbreak. For example, stop distribution and hold the food in locked facilities until tested and released or removed; reject the product at processing or preparation establishments or at ports of entry; remove the food from the premises and reprocess it under supervision; convert the food to animal feed; denature and bury, incinerate, or otherwise destroy the food; or recall all units of the implicated lots. For each product responsible for outbreaks, thoroughly evaluate their health hazard and mode of transmission before reprocessing or converting to animal feed. This is essential to ensure that foods contaminated with pathogens or toxins will not recur in the human food chain and not adversely affect animals. Many processors or caterers whose foods are under suspicion of being a vehicle voluntarily withdraw, and/or cease production and distribution of their products despite the associated financial losses and embarrassment.

Cease processing or preparation of the epidemiologically implicated food. When a vehicle has been identified, cease processing or preparation until corrections are made to eliminate situations that contributed to the outbreak. Ensure that the processes or preparation steps are modified to avoid or minimize contamination; to kill pathogens or inactivate toxins; and to prevent or significantly slow the growth of pathogenic bacteria so that a recurrence is prevented. Control criteria must be established or followed and the process must be monitored with sufficient frequency to ensure prevention of the events that led to the outbreak. Consider implementing a hazard analysis critical point system.

Close the establishment. When imminent risks to health exist if the operations continue, or when contributing factors that cannot be corrected are continuing, close the establishment. Consider reopening only when the contributing factors are identified and corrected or when the operation is brought up to industry standard. Because of legal ramifications, consult with supervisors before taking this action, but the prime consideration must be protection of the public health. Most operators will cooperate if their establishment is identified as the place where epidemiologically implicated foods were processed or prepared, and they may voluntarily offer to close. It may not be feasible, however, to close an operation if a food was prepared for persons residing in an institution, unless an alternate, safe source of catered food is readily available. In such situations, consideration might be given to altering the menu to eliminate high-risk foods

or to altering the operation to ensure safety, such as cooking foods thoroughly and serving them promptly.

INFORM THE PUBLIC

If there is a public health threat, announce the outbreak in the mass media so that the public who purchased the implicated food can be alerted to take action to return it to the place of purchase or other designated location; heat or otherwise prepare it safely; seek medical consultation or treatment; obtain vaccinations; or take prophylactic drugs. Consult with supervisors and medical personnel before taking the last two actions. Record actions on Form N (page 94).

Provide only objective, factual information about the outbreak. Do not release preliminary information that has not been confirmed. The person giving information about an outbreak should be well informed about the etiologic agent being investigated and prepared to deal with questions. If the health hazard warrants a public warning at the hypothesis stage, tell the public why emergency measures are being invoked and that subsequent information may be cause to modify the action. As the investigation proceeds and the etiologic agent is confirmed and contributory factors identified, terminate any emergency measures, and give advice on specific control and preventive measures. Attempt to reach all segments of the population at risk; this may require communication in multiple languages. Route all news releases or statements to all persons involved in the investigation. In situations involving large outbreaks or highly virulent or toxigenic etiologic agents, set up an emergency hotline for the public to call to ask questions. Train staff to handle these calls in a consistent manner so that the advice is the same no matter who gives it.

Regrettably, severe political, legal or economic consequences can result when the public is given faulty information based on poorly tested hypotheses. Such faulty information may be disseminated by the mass media with inappropriate interpretations of the public health significance. Furthermore, this information may be used as an unrealistic base for food programs or food regulations because of either misinterpretations or pressure from misinformed consumer-advocate groups.

CALCULATE ECONOMIC IMPACT OF DISEASE OUTBREAKS

Use data on costs of outbreaks to persuade officials in municipalities, state/provincial governments, national agencies, and legislatures to improve food protection programs by both regulatory and educational efforts; justify foodborne disease efforts of health and food regulatory agencies; and support disease surveillance. Such cost data on different outbreaks can help determine total costs of foodborne disease for a community, state/province, or country.

Try to estimate costs of some outbreaks. In one sense, the expenses of one group (e.g., ill persons) are recouped by other groups (e.g., drug companies, temporary-help agencies) or the group itself later (e.g., settlement

compensations). The standard practice in estimating costs of outbreaks, however, is to document all costs relative to the incidents. Any benefits accrued can be listed separately and subtracted from losses. Some foodborne outbreaks have proven to be very expensive where a large population is exposed to a contaminated food. Businesses and tourism may suffer because of the adverse publicity, and control action may require the implementation of new regulations and educational programs.

Fill in Form O, *Economic evaluation of a foodborne disease outbreak* (page 95), as completely as possible when costing an outbreak. Many costs are not easily available or applicable; however, reasonable estimates relating to specific patients are almost as good as costs when deriving an overall cost of an outbreak (e.g., typical bed-care costs recall figures.) Direct costs are the easiest to measure. The main categories are medical and hospital care; investigation (e.g., epidemiology and laboratory analysis); loss to the food establishment, processor, or business; and productivity losses of ill or infected persons or those caring for ill persons (i.e., usually money not received or work not done for income paid). Estimate income for ill persons. If the exact values are not known, reasonable estimates are still useful because most costs are subject to question. If a daily wage is known, use it. Otherwise, estimate income per day from typical annual salaries for the occupation groups affected divided by 250 (i.e., 5-day work week plus 10 holidays) or another appropriate number. Although an unpaid homemaker has worth, no lost productivity value is normally assigned to this. Get details on food losses from a representative of the food supplier or establishment operator or representatives of insurance companies or legal firms.

Indirect costs are difficult to determine and any figures are subject to question. Though it does not result in financial losses, loss of leisure time is often considered equivalent in value to loss of work time. For retired persons, it could be equivalent to their pension, but they still get their full pension.

If deaths occur, calculate the cost of a life. An average figure can be considered as $500,000 in 1995 U.S. dollars. Young children and elderly persons are normally calculated as being worth less than persons of wage-earning age. Deaths, however, are seldom attributed entirely to foodborne disease agents; other underlying causes often contributed to these deaths. For example, if an elderly or debilitated person suffers from foodborne illness, the fatality may be only 50% attributable to this syndrome. If death certificates are available, review these to decide the primary cause of death. However, information on these is often incomplete, and estimates can be made without them.

Even if some information is lacking, complete as much of the form as possible. Rational estimates can substitute for unknown data as long as the qualification is mentioned.

SUBMIT REPORT

Summarize investigative data in a narrative report. State the case definition and methods used to gather clinical and exposure histories (e.g., telephone interview, questionnaires). Describe situations that led to contamination of the

food, survival of the etiologic agent, and proliferation or concentration of the agent up to the time of consumption. Include all events that contributed to the outbreak to guide control and preventive measures. State control actions taken or recommended, recommended preventive measures and the effectiveness of these actions (Form N, page 94).

Compare your data with the listings in Tables B (page 100), D (page 125) and E (page 132) before assigning the etiologic agent and the vehicle. Outbreak confirmation is based on time, place, and person associations; recovery of etiologic agents from specimens, cases and samples of food; identification of sources and modes of contamination; means by which pathogens or toxic substances survived treatment or proliferated. All of these, however, might not be found in any one investigation.

Complete Form P, *Foodborne illness summary report* (pages 96, 97). Attach the narrative and the epidemic curve. Also attach Forms D2, G, H, I, J, K, M, and N and other data that will provide supplemental information to reviewers.

Send this report through administrative channels to the appropriate agency responsible for foodborne disease surveillance at state/provincial and national levels. Make the final report as complete as possible, so that the agency can accurately interpret the results and develop a meaningful foodborne disease data bank. Such a complete report is useful in the event of litigation. In interest of continuing cooperation, give all participants in the investigation due credit and send each a copy of the report. Also, send copies of the report through administrative channels to agencies that have jurisdiction over the implicated food, initiated the alert, and participated in the investigation. Consider publishing investigative data of important outbreaks (e.g., large outbreaks; unusual outbreaks, etiologic agents, or vehicles; outbreaks with associated mortality; new agents) in peer-reviewed journals.

Those concerned with food protection and with public health should make every possible effort to ensure the complete investigation and reporting of foodborne diseases. Without reliable, complete information, the trends in foodborne disease, incidence, and causal factors of the disease are difficult to determine. Good surveillance is essential for detecting and evaluating new foodborne disease hazards and risks.

USE OUTBREAK DATA FOR PREVENTION

The primary purposes of a foodborne disease investigation are to identify the cause, establish control measures, and take actions to prevent future illness. Control measures can be effected either at the time of the investigation or immediately afterward by identifying a contaminated or otherwise hazardous product and removing it from the market.

To decrease incidence of foodborne illness, it is necessary to identify causal factors, develop practicable preventive procedures, and communicate them to those who can put them into practice. Factors shown by experience to contribute frequently to outbreaks are cited on pages 30–34, in Table B (page 100–122) and Keys A–H (pages 133–140). Inform managers, employees, and homemakers (as applicable) of the circumstances that contribute to

outbreaks, and instruct them in proper food processing, preparation, and storage procedures.

Survey establishments that process or prepare similar foods to see whether conditions that contribute to outbreaks of illness are widespread. If so, initiate an industry-wide training program. If education fails to achieve the desired results, take other actions (such as targeting high-risk establishments with more frequent evaluations, conducting hearings, seizing contaminated lots, and prosecuting violators) to correct hazardous operational procedures. After such actions are taken, periodically evaluate these establishments to verify whether faulty procedures have been corrected or reintroduced into the operation, and to verify that critical control points are under control and being monitored effectively. If not, take appropriate educational and corrective action.

Alert the public to hazardous conditions that can affect them and motivate them to become concerned about their food supply. Only then will they insist on wholesome, safe foods that are processed and prepared in sanitary establishments. Consider establishing a newsletter containing reports that describe outbreaks, contributing factors, and preventive measures. Distribute copies to physicians, hospitals, the food industry, and others interested in foodborne illness and its prevention.

Foodborne illnesses are preventable, but prevention requires that those in the food industry and in health and regulatory agencies be constantly vigilant to ensure that the hazards are understood and questionable operating procedures are avoided. Therefore, implementing a hazard analysis critical control point system can provide high assurance of food safety. This system is based upon: detecting hazards from epidemiologic data and observations of operations; identifying critical control points; establishing control measures, criteria, and critical limits; monitoring the critical control points to ensure that the process is under control; and taking immediate action whenever the criteria are not met. This is the subject of another IAFP manual, *Procedures to Implement the Hazard Analysis Critical Control Point System.*

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