Chapter 32

OUTBREAK INVESTIGATION

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INTRODUCTION

The use of the epidemiologic method to identify and control an acute disease problem in a population represents the most dramatic application of the science of epidemiology. Knowledge gained from a well-conducted outbreak investigation can enable the investigator to intervene and control an epidemic. The relatively quick tempo of this scientific endeavor also contributes to its unique place in research. The literature is replete with classic examples of outbreaks occurring in military populations.[1–3] Carrying out the military mission is associated with living, working, eating, and playing in a group environment under harsh conditions. This togetherness, coupled with potentially unique occupational and environmental exposures, can result in outbreaks in deployed service members. Vaccines or effective chemoprophylaxis do not exist for many endemic diseases. Recognition of new pathogens and the reemergence of known pathogens with new drug resistance patterns have contributed to a resurgence of infectious diseases as a threat to the military force in the field.[4–9]

The Epidemic Intelligence Service program for public health officers at the Centers for Disease Control and Prevention (CDC) grew out of the fear of biological warfare during the Korean War.[10] As the 21st century opens, the potential for governments or individual terrorists to use biological or chemical agents against a population has not lessened and may indeed be greater than it was in the 1950s. The 1995 terrorist attack on a Japanese subway train using the nerve gas sarin resulted in more than 5,000 people injured and 11 deaths.[11] Biological warfare and biological terrorist events by definition seek to create epidemics and so the need remains for a cadre of capable epidemiologists to respond to domestic or military public health emergencies of this nature.[12]

This chapter is intended to provide a practical, detailed discussion of the specific steps to use in conducting the investigation of an outbreak in the field (Exhibit 32-1). The initial purpose of the investigation is to determine the population at risk and identify the specific cause or exposure risk factors of disease. As the description of the outbreak becomes clearer, explicit objectives can be formulated for the planned field work to develop and test hypotheses proposed to explain the outbreak. This permits effective intervention, which in turn will end the crisis.

EXHIBIT 32-1

OUTBREAK INVESTIGATION METHODOLOGY

- Verify the diagnosis or define the problem
  - Establish a case definition
  - Ascertain cases
- Verify the existence of an epidemic
- Organize a multidisciplinary team
- Identify disease control measures
- Describe the outbreak by person, time, and place
- Identify risk factors and mechanisms
- Develop tentative hypotheses and a plan
  - Determine type of outbreak and critical exposure
  - Hypothesize about mechanism of transmission
  - Develop survey instruments
  - Plan for administrative and logistical considerations
  - Identify medical treatment resources
  - Reevaluate control measures in place
- Test hypotheses
  - Collect data and specimens
  - Determine the need for additional studies and analysis
  - Evaluate the adequacy of the case definition
  - Establish criteria for deciding outbreak is under control
- Compare results to those of the published literature
- Finalize report with control recommendations
  - Write an executive summary and prepare a complete report
  - Evaluate effectiveness of control recommendations

VERIFY THE DIAGNOSIS OR DEFINE THE PROBLEM

An outbreak investigation is an iterative process that passes through distinct phases. An outbreak investigation begins with the recognition of a problem, usually cases with similar symptoms clustered in space and time. An alert observer must then call on the services of the public health system. The appointed investigator needs to obtain as much detailed information as possible from the initial report. Cases of clinical illness should be fully described, to include the type of symptoms, frequency of symptoms, onset time, and duration of the illness. In some instances, the actual diagnosis is already known. On other occasions, only a collection of signs and symptoms is apparent. When the etiology is unclear, the differential diagnosis for the clinical presentation of symptoms and physical exam findings must be considered. Clinical clues and timing of onset can help narrow the possibilities. For example, the presence of blood and fecal leukocytes in diarrheal stools indicates an inflammatory process and limits the number of pathogens to be considered. The presence of paresthesias or other neurological symptoms immediately suggests the possibility of a chemical intoxication from ciguatoxin, scombrotoxin, paralytic shellfish poisoning, or mushroom poisoning. It is very important at this early stage to determine the type of studies (eg, smears, cultures, serologies, radiographs) necessary to verify the diagnosis. Appropriate laboratories should be made aware of the possible organisms, toxins, or other agents being considered because some will require special media or special testing.

Following this initial notification of a problem, two equally important determinations must take place: (1) verification of the diagnosis or at least definition of the problem and (2) verification of the existence of an epidemic. Often these two fundamental steps will occur simultaneously. The order in which these two steps are listed in the investigation process will vary, depending on the leader's preferences. Part of the complexity of launching and conducting an investigation of this type is the necessity to organize and take action in multiple directions simultaneously. Delay in taking action could result in loss of important data.

Establish a Case Definition

After gathering the initial impressions, the investigators must develop a workable case definition and begin case finding efforts. Simple, objective criteria are best. The type, magnitude, and frequency of symptoms, as well as their duration, must be quantified. Categorization of cases as possible, probable, or confirmed using type and number of symptoms, culture results, and other pertinent information will sometimes be useful in further refining the case definition. Case definitions for known diagnoses have been published by the CDC,[13] and the use of standard definitions allows for better comparisons between outbreaks. The case definition criteria must be applied consistently to all persons studied. A broad case definition may be refined later but a very narrow initial definition may miss cases. Consider the clinical spectrum of apparent illnesses being seen and whether this is a disease or condition in which a large number of asymptomatic cases are likely. Arriving at a clear case definition may sometimes be extremely problematic, as is best exemplified by the attempts to establish a case definition of "Gulf War syndrome."[14] An accurate and sensitive case definition is essential to reduce misclassification and confounding when investigating potential exposure risk factors.[15] Epidemiologic investigation of outbreaks usually begins with identification of a distinct disease syndrome and proceeds to evaluation of risk factors using epidemiologic methods. This is successful even with newly recognized diseases such as toxic shock syndrome.[16] Studies of Persian Gulf War veterans have been complicated because neither a distinct clinical picture nor distinct exposure risks are clear.[17]

Ascertain Cases

Case finding efforts begin using the case definition or definitions established. Investigators must start and maintain a line listing or log of all presumed cases. This log should include name, social security number, unit, sex, age, and other relevant information (eg, pending culture results). It is important to include a phone number or contact location for each presumed case on the list. Other possible sources of information for finding cases with the condition of interest should be considered. These sources might include medical records, emergency room logs, specific clinic logs, or laboratory records. A check on relevant quality control or quality assurance procedures in the laboratory, clinic, or other facilities should be done to rule out artifactual increases in numbers. On military installations, the surrounding civilian community should be checked for additional cases through queries to local physicians, clinics, or public health departments. Because of the mobility of the military population, cases may have been exposed in one location and have traveled elsewhere, even across continents, during the

incubation period. Cases of coccidioidomycosis, in a classic example, occurred as the result of exposure on military bases in endemic areas in California but did not present until personnel traveled elsewhere.[18] In situations such as these, distant locations may need to be contacted. The need for a thorough travel history and an elevated level of diagnostic suspicion is key to case recognition. The case finding efforts may need to be broadened to the Offices of the Surgeons General in the Department of Defense and to the CDC. Patients may even self refer once news of the outbreak and the investigation becomes known. The media can assist health authorities in informing the public and in directing the referral of possible cases for evaluation through its public service announcements. In military settings, cases may sometimes be found by using questionnaires to screen units. Follow-up interviews and evaluations may be required to determine actual case status. For communicable diseases, finding the contacts of known cases may identify additional cases and provide other relevant information. Unrecognized cases, those with mild illness or who are asymptomatic, may provide additional clues or supporting data concerning the presumed disease or hypothesized etiology.

VERIFY THE EXISTENCE OF AN EPIDEMIC

After describing, defining, and finding cases, then the question is whether the number of cases found constitutes an epidemic. Last defines an epidemic as the "occurrence in a community or region of cases of an illness… [or] health-related behavior or…event clearly in excess of normal expectancy.[19p54] A single case of an extremely rare or exotic disease, such as botulism poisoning, pulmonary anthrax, or Ebola infection, also constitutes an epidemic (and may indicate a biological terrorist event). To determine the usual level of disease occurrence, investigators must examine available historical data, such as medical records, clinic logs, and laboratory logs. Personally visiting sites such as the records department or the laboratory may lead to other useful data sources (eg, culture logs by body site of specimen source). Site visits will also provide a better understanding of the type and quality of the data available. To conclude that the current level of disease is excessive, investigators must first define the event and then compare the current rate with that of the past. Comparing the routine surveillance rates for skin disorder consultations for British troops in Bosnia disproved media reports of a serious outbreak.[20] The significance of possible differences can be depicted graphically and assessed statistically by testing differences of proportions or calculating confidence intervals.

The limitations of the data or the data source affect the interpretation of the data. The ideal or preferred morbidity measures for disease ascertainment should also be identified. The profound changes in the practice of medicine precipitated by the managed care movement make comparisons with historical rates problematic. Changes in access to care, referral patterns, test-ordering practices, hospitalization decisions, utilization management mandates, and other health management procedures can all affect the apparent incidence of disease.[21] These changes in practice patterns decrease the utility of historical data, such as hospitalization rates, for assessing secular trends and identifying outbreaks. Investigators must judiciously determine if the epidemic is real or artifactual after assessing the situation, data sources, and practice patterns.

Pseudoepidemics can also occur because of false-positive results of laboratory tests or from changes in personnel or administrative processes affecting the sensitivity of diagnosis. In the 1980s, an apparent outbreak of skin cancer occurred at Letterman Army Medical Center, as determined by inpatient admission statistics. However, it was an administrative policy change directing that all patients with skin cancer be admitted for biopsy and excision that led to this artifactual epidemic (Kadlec RK. Walter Reed Army Institute of Research, 1989. Unpublished data). Specimen contamination in the microbiology laboratory has also been implicated in a number of "clusters" of multidrug-resistant tuberculosis. Newer techniques of molecular biology, such as pulsed-field gel electrophoresis (PFGE), may be used to detect these pseudo-outbreaks and document their extent and resolution.[22,23] The risk also exists of cross contamination in the molecular laboratory from contamination of polymerase chain reaction assays. The most serious dangers are contamination of specimens with postamplification products from previous analyses or contamination of negative specimens with controls or positive specimens.[24] Emphasis on new and expanded surveillance programs for emerging infectious diseases has already been demonstrated to increase the potential for identifying pseudo-outbreaks, as occurred with cyclosporiasis in Florida and cryptosporidiosis in New York.[25] It is advisable to get confirmation by an appropriate reference laboratory early in any investigation of apparent clusters of emerging pathogens (see chapter 34, Laboratory Support for Infectious Disease Investigations in the Field).

ORGANIZE A MULTIDISCIPLINARY INVESTIGATIVE TEAM

After the initial assessment has verified the diagnosis (or at least delineated the problem) and verified the existence of an epidemic, a multidisciplinary investigative team should be organized. The team may be organized from the staff available locally or may be a consultant team of experts invited to travel to the location of the epidemic. The following guidance can be applied to either a small local group of public health workers or to a large, experienced consultant team.

Composition of the Team

The optimal composition of the team by type of expertise is key. Epidemiologists do not work alone, and cooperation between disciplines is essential to the success of the investigation. Physicians and nurses trained in preventive medicine need the expertise of statisticians and scientists in sanitary engineering, environmental science, industrial hygiene, health physics, entomology, microbiology, or toxicology, as the situation dictates. Clinical expertise in such areas as infectious diseases and neurology may be added, depending on the problem being investigated. Veterinary public health experts are critical to any investigation of possible foodborne disease outbreaks or zoonotic outbreaks. Appropriate laboratory expertise must be present from the outset to ensure the correct specimens are obtained and properly processed and that appropriate diagnostic testing is used. New techniques in molecular biology may be incorporated into the laboratory investigation, often at an off-site reference laboratory. Addition of someone with medical informatics expertise will facilitate the proper planning for data entry, computer programming, and automated analysis of investigation results. Software packages designed for outbreak investigation (eg, Epi Info, CDC, Atlanta, Ga) may expedite data management and analysis. Frequently, the programming and analysis phase of the investigation will continue long after the field portion is completed. Logistic or supply experts may be needed for large, complicated operations, especially those conducted outside the United States. A public affairs representative or a media spokesman should be identified and made an integral part of the investigation team. For teams of outside experts, assignment of a key local staff member to be a liaison with the investigation team should help ensure command access and support.

The team leader, who is responsible for organizing and conducting the investigation, should be specified. The nature of the problem, the complexity and sensitivity of the situation, and the expertise, experience, and availability of potential leaders will determine who is given this responsibility. An additional senior person with experience in outbreak investigation may be identified to serve as an off-site consultant to the operation. The team leader should report daily to this consultant, and the consultant can help update other consultants, agencies, and commands outside of the local jurisdiction. This will decrease demands on the time of the team leader. The senior consultant is also vital to obtaining any additional support (eg, personnel, equipment, supplies, references) required for the investigation, but this should not occur independently of the normal chain of command.

Logistical Plans and Management

The supplies needed by both local and outside investigative teams are similar, but logistical support planning is more complicated for a nonlocal team. This section will discuss some of the logistical issues that outside teams face. Documents required in preparation for team travel may include government orders, country clearances, passports, international driver's licenses, immunization records, powers of attorney, credit cards, and property passes. Specific arrangements must be made for transportation of equipment and personnel onsite and lodging of the team. Members of the group may need additional immunizations and chemoprophylactic medicines, as well as supplies of their personal medications. Diagnostic, laboratory, and automation equipment are usually essential. Supplies for human specimen collection and environmental specimen collection may be available locally; if they are not, they must be brought in by the team. Published references that discuss collection of laboratory specimens in specific types of outbreaks should be consulted.[26] Questionnaires, blank rosters, preprinted labels, key phone numbers, cellular telephones, beepers, laptop computers, software programs, scanners, portable photocopy machines, digital cameras, and any other relevant specialized equipment should be included. Statistical references and disease-specific reference material are also important. Exhibit 32-2 lists a basic supply package designed for the Army's Problem Definition and Assessment team, which must be able to deploy to the site of a public health crisis within 24 hours. The supply chain to be used for any future require-

EXHIBIT 32-2

CONTENTS OF PROBLEM DEFINITION AND ASSESSMENT TEAM KIT

Bag No. 1: Medical Equipment

- Stethoscope, sphygnomanometer, otoscope/ophthalmoscope (1 each)
- Extra bulbs for otoscope/ophthalmoscope
- Extra batteries for othoscope/ophthalmoscope (4 large)
- Tongue depressors (50)
- Tempa dots (box of 100)
- Penlight (1)
- McArthur microscope and attachments (1)
- McArthur microscope instructions (1 set)
- Extra batteries for McArthur, size AA (8)
- Microscope slides, frosted end (300)
- Cover slips for slides (300)
- Immersion oil, 1 bottle
- Lens paper
- Slide holding box (2)
- Calculator, solar powered and case (1)

Bag No. 2: Blood or Stool Specimen Collection Supplies

- Vacutainers, 13 cc red top with silicone separator (100)
- Vacutainers, 7 cc green top (50)
- Vacutainer holders and tourniquets (6 each)
- Multidraw vacutainer needles, 20 g (125)
- Syringes, 20 cc (5) and hub needles, 21 g (10)
- Alcohol swabs and 2x2s (200 each)
- Band-Aids (100)
- Plastic Serum transport vials, 5cc (200)
- White labels, silk-type, marked PDA (200)
- Labels, silk-type, marked PDA (200)
- Polyethylene specimen bags, ziplock type (25)
- Perma markers for labeling specimens and plastic vials (6)
- Stool cups, carton-type (20)
- Sterile urine cups, plastic-type (20)
- Culturette, throat swabs (20)
- Biohazard bags (10)
- Gloves, 7 1/2" & 8 1/2" (10 each)
- Ammonia inhalant capsules (10)
- Parafilm sealant paper (1 roll)
- Filter paper for PCR of blood samples
- Sharps disposal boxes
- Centrifuge
- Transfer pipettes
- Freezer boxes
- Boxes to store and ship plastic vials

Bag No. 3: Bacteriology and Parasitology Supplies

- Cary-Blair media in REMEL plastic tubes (50)
- Buffered glycerol saline in REMEL plastic tubes (50)
- PVA fixative in REMEL plastic tubes (50)
- 3 cc syringe with 24 g (or 25 g) needle for leish aspiration (25)
- Blades, scalpel type for scraping of lesions
- NNN culture media in slants (20)
- Schneider's media in slants (20)
- Gram stain kit (1)
- CAMCO giemsa quick stain (1 bottle)
- Diff quick stain set (1)
- Methylene blue stain (1 box of squeeze type)
- Stain jars (3), forceps (1) and paper towels (1 small roll)
- Distilled water, 100 cc (1 bottle)
- Sterile saline, 5 cc (10 glass vials) for leish aspiration (*without* Na Azide)
- Normal saline in dropper bottle (1)
- Methanol, 100 cc (1 bottle)
- Plastic squeeze type bottles for use in washing slides, 100 cc (2)
- Na Azide, 15%, preservative for serum preservation (1 bottle)
- One set of Gram stain and Diff quick staining instructions
- One set of REMEL kit instructions
- One set of leishmania aspirate/smear instructions

Bag No. 4: Forms and Administrative Supplies

- Rubber bands (1 bag)
- Notebook and notepad (1 each)
- Pencils, sharpened (20)
- Questionnaires, postdeployment type (400)
- Questionnaires, febrile illness type (100)
- Clinical flow chart and lab sheets (20 each)
- Medical surveillance report forms, two-sided (200)
- Daily and weekly medical surveillance summary forms (200 daily, 50 weekly)
- Medical surveillance instruction sheets (50)
- PDA team booklet (1 copy)
- Laptop Computer (1) with Case
- Portable printer (1) with printer paper

ments should be determined. Local administrative support for generating rosters, typing, photocopying, or just answering the phone should be requested before the team arrives. The local laboratory should be consulted about the team's need for work space, laboratory space, centrifuges, freezer space for specimens, dry ice, liquid nitrogen, and shipping assistance. Any local staff support needed for key tasks such as venipuncture and aliquotting should also be discussed. The extent of local support available may affect the size and composition of the team and the need for off-site laboratory support. The team leader should determine which reference laboratory will be used for more sophisticated requirements.

Leadership and management skills are key to the success of the scientific investigation. It is critical for the team leader and team members to conduct the investigation in a calm, methodical manner even though decisions may need to be made quickly and actions taken promptly. Team members must resist the impulse to jump to premature conclusions in response to the intense pressure to solve the problem immediately. The possibility of a second wave of cases should be kept in mind before the team quickly declares the outbreak over. If new cases do continue to occur, the pressure on the team to find the cause and end the outbreak will dramatically increase. Departure dates should not be set until it becomes apparent how both the epidemic and the investigation will unfold.

The leader must assign areas of responsibility to team members in accordance with their expertise and identify locations for work space. A list of phone numbers and beeper numbers for team members and work sites should be established and exchanged. A time and location for team meetings should be determined. These should be held at least daily and probably twice daily initially. The leaders should make a task list with assignments for follow-up and track status reports at subsequent meetings. All decisions made at these meetings, such as case definitions to be used or source and type of controls needed, should be recorded.

The investigation should be organized both with the big picture in mind and with very close attention to the details—the details may provide important ideas. It is also essential to be tactful when obtaining detailed information because the existence of an epidemic is a source of potential embarrassment. Some epidemics will have major economic and political ramifications. Careful judgement about the release of preliminary results is thus vital. Diplomacy will be needed to establish and maintain cooperation from the many groups within the community.

A local staff member must be put in charge of media relations and determine what information has already been given to the press. The team leader should not be the spokesperson. The public affairs director should attend team meetings and ensure all press releases are cleared by the team leader and local command authorities. Likewise all news releases and statements to the press should be given to each member of the investigative team. Only objective, factual information should be released; the release of preliminary information should be avoided. The rationale for any emergency control measures should be explained. Some situations may warrant setting up an emergency hot line to answer questions. Hot line operator staff must be trained to provide consistent information to all callers.

The military chain of command and the responsible public health authorities do not change when an outbreak occurs. Authority to ban food sources and to close dining facilities, swimming pools, operating rooms, and other such facilities remains with the local commander acting on the guidance of the local preventive medicine officer. The investigation team serves as a consultant and makes recommendations to the local commander. Investigation team members are subordinate to the designated team leader. Local preventive medicine staff should be integrated functionally with the investigation team to conduct the investigation. The team leader should have an initial in-briefing with the local commander, provide interim updates as the situation dictates, and provide an out-briefing before the team leaves.

IDENTIFY DISEASE CONTROL MEASURES

When feasible, disease control measures should be implemented immediately after determining the nature of the problem and that an epidemic is ongoing. The initial control measures are intended to keep the outbreak from spreading and to limit its impact on the population. Examples of control measures prescribed to individual susceptible persons at risk would include the use of immune globulin during hepatitis A outbreaks, use of rifampin as chemoprophylaxis for meningococcal meningitis, use of penicillin to prevent streptococcal disease, and initiation of case isolation or contact notification. Examples of general control measures include reinforcing handwashing recommendations and

changing food preparation processes. In some situations, seizing raw materials (eg, drugs, food), closing an establishment (eg, restaurant, pool), or issuing an advisory to boil water may be warranted. Environmental interventions, such as mosquito or rodent control, may also be indicated. Control measures should be tailored to the situation and may be as simple as restricting movements of the population at risk or temporarily discontinuing new trainee arrivals, as was required by varicella outbreaks in at the Defense Language Institute at Lackland Air Force Base in San Antonio, Tex.[27] Implementation of control measures will often require coordination with other agencies, such as major commands, civilian health departments, or the Food and Drug Administration (to hold or recall commercial items). The effectiveness of any disease control measures implemented by local health authorities or recommended by the investigation team will need to be evaluated as the investigation proceeds. Control measures should then be added or the existing ones altered, as indicated by the evaluation.

DESCRIBE THE OUTBREAK BY PERSON, TIME, AND PLACE

Studying the distribution of the disease or condition in the affected population begins with a detailed description of the outbreak in terms of person, time, and place. Hippocrates was among the first to note the importance of this triad in his paper *Air, Waters, Places*.[28]

Orient the Outbreak to Person

The first task is to identify the population at risk. This is done by characterizing the population members by age, race, sex, occupation, military unit, or other demographic grouping. If the outbreak appears to be associated with a special event, rosters of attendees must be obtained from the event organizers. Within this population, the nature of the risk may be seen in the presumed intensity of exposure. The likelihood of transmission should be considered both within and beyond this population. Noting who has been spared often provides important clues for formulating a hypothesis on the cause of the outbreak. Factors affecting the population at risk to be considered include the extent of migration into and out of the group.

Subjects for evaluation must be selected from within the population at risk. These should include both cases of the disease under investigation and controls or persons with no symptoms of disease. Both cases and controls should have had the opportunity for exposure to suspected risk factors. Controls should also be susceptible to the condition of interest. The team must decide if it will study all of the targeted population or just a particular subset or sample. A method will have to be decided on to choose an appropriate sample, depending on the circumstances of the outbreak and the population (see chapter 33, Epidemiologic Measurement: Basic Concepts and Methods). Alternatively, investigators could select one or more matched or unmatched controls for each case.

Although it is not the job of the team to provide clinical care for cases, it is of the utmost importance for clinician team members to conduct a personal interview and evaluation of at least a few cases in the early phase of the investigation. This will assist in verifying the diagnosis and in subsequent planning of specific objectives for data collection and analysis. Team members should ask those being interviewed what they think caused the outbreak, since they may have surprising insight and will frequently provide additional, pertinent information.

Attack rates of disease in the population at risk should be estimated based on the interview data from cases, providers, and others. Rates of disease by substrata are also very useful. Investigators should always examine attack rates by militarily relevant groupings, such as unit, barracks, training site, military job specialty, and rank (eg, officer versus enlisted). In foodborne outbreaks, attack rates for those who did and did not eat specific food items will be used for formal hypothesis testing.

Orient the Outbreak in Time

Viewing graphic displays of epidemic curves or flow charts may yield important conclusions about the outbreak, such as whether it is a common source event or whether transmission is ongoing. Correlating both time and place on spot maps can show secular trends of waves of illness moving across a community. The epidemic curve should be plotted using a histogram to quantify the number of cases; this will graphically display the outbreak. Cases meeting the previously determined case definition are plotted by date of symptom onset, with the X axis depicting time and the Y axis showing number of cases. The first case recognized is sometimes referred to as the index case if this case is thought to have introduced the organism into the population. From this curve, the incubation period of known

diseases can be used to determine a range estimate for the time of exposure. Alternatively, the incubation period of an unidentified disease may be calculated from the time of symptom onset and a time of known probable exposure from a unit event. Other important characteristics of cases can be annotated on a histogram of the epidemic curve by designating specific symbol notations to indicate cases who are asymptomatic, work as food handlers, have positive cultures, or have died. Arrows may be used to note on the graph the arrival of the investigation team and the application of any control measures. A frequency polygon is sometimes used instead of a histogram to depict distribution of data from two or more categories.

Investigators must assess evidence for transmission patterns from the time distribution of cases. Certain patterns indicate a specific type or mode of transmission. Figure 32-1 depicts a common source outbreak of *Shigella dysenteriae* infection among hospital staff members.[29] Common source epidemics occur when a population is exposed to a pathogen spread by a vehicle such as food or water at a single event or within a short period of time. Cases occur rapidly after the first onset, reach a peak, and then decline because of the relative uniformity of the incubation period. The rapid rise with a tight temporal clustering of cases and subsequent fall of the epidemic curve is compatible with a point source. The epidemic curve of a common source epidemic follows a log-normal distribution. The median incubation period for a common-source, single-event epidemic can be determined by finding on the histogram the time at which 50% of the cases have occurred. The approximate time of infection can be determined by subtracting the average incubation period in hours or days from the time at which the median case is located on the epidemic curve.

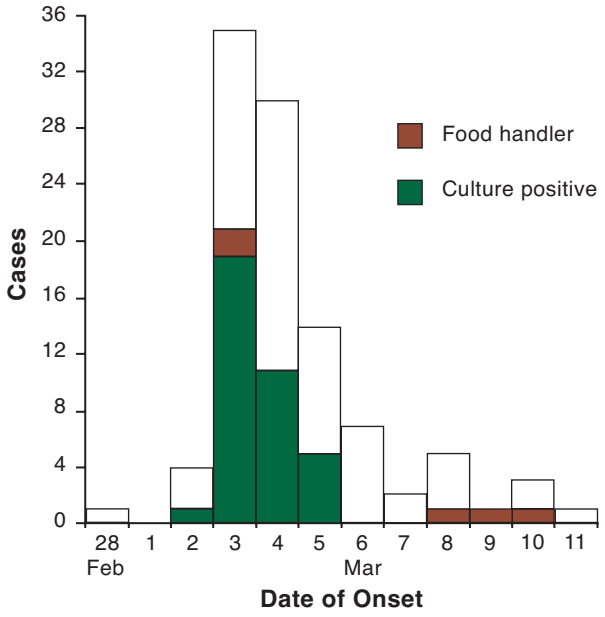


Figure 32-1. This is a graph showing the cases of hospital-associated infection with *Shigella dysenteriae* by date of onset and culture result. (Four cases with unknown date of onset have been excluded.) These cases occurred in the Bethesda Naval Hospital, Maryland. No index case was identified. Five food handlers with illnesses meeting the case definition had onset of symptoms concurrent with other cases.
Source: Centers for Disease Control. Hospital-associated outbreak of *Shigella dysenteriae* type 2—Maryland. *MMWR*. 1983;32:250–252.

In some outbreaks, the common source may continually or intermittently expose the population, resulting in an epidemic curve from multiple exposures at different times. The distribution of cases will continue over a protracted period of time, and interpreting the curve will be more complex. These outbreaks may be referred to as a common-source, multiple-event epidemic. Epidemics resulting from person-to-person transmission will be reflected in an epidemic extended over a number of incubation periods. The curve will show a clustering of cases but with a relatively gentle upslope, and, after several generations of cases, an eventual decline in cases will occur. Figure 32-2 depicts a person-to-person outbreak of respiratory disease at Fort Leonard Wood, Mo, with seasonal variation and the effect of bicillin applied as a control measure.[30] An epidemic curve can have a mixed pattern if the initial transmission occurs via exposure to a common source but subsequent transmission is person-to-person.

Creating flow charts is useful to trace a chain of infection associated with the cases. Concentrating on the earliest cases may help pinpoint the precipitating event. Determining who the earliest cases were may help explain how the disease was introduced into the setting. Also, evaluation of unusual or atypical cases may provide additional clues. But investigators must beware of red herrings. In the shigella outbreak depicted in Figure 32-1, a hospital staff dining facility was implicated as the source. However, case ascertainment identified one culture-positive case with the same uncommon strain who had no exposure to the staff facility but had eaten at a small cafe in the same institution. No other cases were traced to that cafe. On additional questioning, the cafe staff remembered borrowing lettuce and tomatoes at the end of the day from the implicated

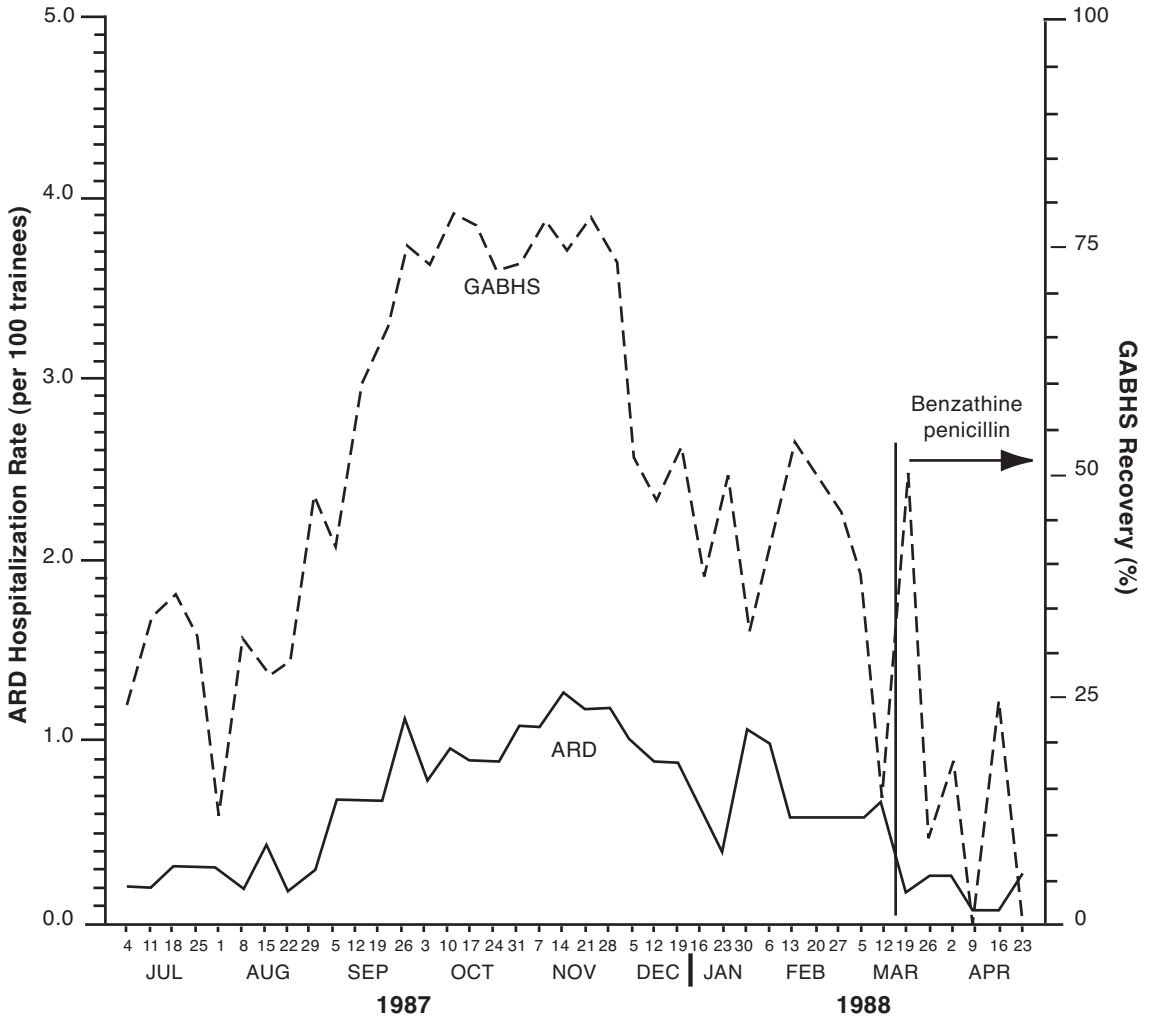


Figure 32-2. This graph shows the rates of hospitalization for acute respiratory diseases for Army trainees at Fort Leonard Wood, Mo, from July 1987 through April 1988. The dashed line represents the percent of recovery of group A β-hemolytic streptococcus (GABHS) from the trainees. Prophylaxis with benzathine penicillin was started in March 1988 as a response to the outbreak and is noted on the graph. GABHS recovery rapidly declined following initiation of benzathine penicillin prophylaxis, which effectively controlled the outbreak. The acute respiratory disease rate also declined following initiation of penicillin prophylaxis.
Source: Centers for Disease Control. Acute rheumatic fever among Army trainees—Fort Leonard Wood, Missouri, 1987–1988. *MMWR*. 1988;37:519–522.

dining facility. Further analyses implicated salad served at the dining facility as the vehicle of transmission. Exhibit 32-3 lists possible reasons for exposed persons not becoming ill and for nonexposed persons who appear ill.[31]

Evidence must be assessed for chronological distribution of disease. Recurrent cycles of epidemics allow investigators to calculate the generation time. Seasonality may affect rates of certain diseases such as influenza. Investigators must be careful interpreting secular trends, however, because small numbers may affect data analysis. Disease rates characterized by time may also vary with the provider's enthusiasm or the resources available to help detect and report disease. Changes in personnel, clinical practices, reporting procedures, data collection forms, or case definitions may all alter the apparent rates of disease.

Orient the Outbreak to Place

The pattern of disease should be described by determining the location of quarters, work, and recreation and noting geographic distribution of cases by creating a spot map. The dimension of time can be added to place with the use of colored pins. Biological,

EXHIBIT 32-3

POSSIBLE EXPLANATIONS FOR INACCURATE MEASURES OF ASSOCIATION

Endemic background cases

Errors in case definition

Individual susceptibility and immune status

Exposure to insufficient dose and inoculum

Cross contamination between potential vehicles

Exposure to vehicle contaminated in another way

Misclassification of exposures

- Technical errors
- Untruthful or inaccurate responses

Secondary person-to-person transmission

Adapted with permission from *Guidelines for the Establishment of Systems for the Epidemiological Surveillance of Food-borne Diseases and the Investigation of Outbreaks of Food Poisoning*. Pan American Health Organization. Division of Communicable Diseases Prevention and Control. 1993.

chemical, physical, or climatic factors affecting the environment should be included. Geographic information software may help plot space-time characteristics. Preparing and maintaining an outbreak investigation kit with supply items routinely needed for most environmental investigations will assist investigators in performing timely on-site evaluations.

Investigators should consider case clustering by source of food, water, milk, ice, or shellfish. If foodborne disease is under consideration, an on-site inspection of all facilities potentially associated with the outbreak is appropriate. Any problems in air quality, food sanitation, water sanitation, or hygiene practices should be identified. Utensils, equipment, filters, and surfaces used in preparing the food or other suspect vectors should be cultured. Leftover foods should be set aside for possible laboratory analysis.[32] Water should also be considered as a possible etiologic agent at the outset. Too often water and ice are only considered as a potential source of contamination after food has been ruled out as the cause. The time factor is crucial to ensure rapid collection and culture of food, water, ice, and other environmental samples before food or ice have been consumed or thrown away or water lines have been superchlorinated. In a large outbreak of campylobacteriosis in 1990 at Fort Knox, Ky, the investigation team hypothesized that sludge and dead birds found in a water storage tower were associated with infection. The tank was disinfected and refilled before culturing of the suspect water and sludge could be accomplished.[33] Investigators always look for inadequate health practices, such as improper handwashing or food handling practices and recent procedure changes; the adequacy of training and supervision of workers should also be evaluated. Microbiological or chemical contamination can occur at numerous points, and investigators may need to examine multiple sequences of events or look for a unique order of events to find the cause. Correlation of time and temperature at each stage of food processing should help identify the critical point.

Samples of water should be collected directly from the source, from storage tanks, and from high and low points of the distribution system, in accordance with standard methods.[34] Characteristics to be measured include temperature; pH; turbidity; and free, combined, and total residual chlorine. Bacterial examinations for potable water should include total organisms of the coliform group, indicative of fecal contamination, and a standard plate count (heterotrophic plate count) because large bacterial populations may suppress the growth of coliforms. Turbid waters may contain particles with embedded bacteria protected from contact with chlorine. This may also contribute to coliform masking. The absence of coliforms does not ensure the absence of viruses, protozoa, or helminths, which may be more resistant to chlorine treatment than fecal bacteria. Norwalk virus and the related small round structured viruses, which are very resistant to chlorine, are a major cause of acute nonbacterial gastroenteritis in adults.[35] Protozoa such as *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium* species have also been implicated in waterborne outbreaks.[36] Plumbing cross connections between potable water and contaminated water, loss of positive line pressure, line breaks, and line repairs may allow bacterial contamination. Spot maps of water distribution lines and points may provide insight on potential areas for contamination.

The on-site inspection is vital to the environmental evaluation of any outbreak associated with a military field setting. A broad knowledge of military food technology and field water supply options is essential for preventive medicine staff. Investigators must carefully identify all local sources of food and all types of rations served in the field: A, B, T, or Meals Ready to Eat (MREs). Food purchased from the local market in foreign countries may re-

quire specific washing and disinfection before consumption. MREs, a foil-wrapped ration packaged in a laminate pouch, have been the main operational ration used by the Department of Defense since they replaced the C-ration in 1983. Although MREs have been found to be extremely safe, contamination could occur at the manufacturing and processing plants or if a break in package integrity occurs. Investigators must determine the lot number, storage temperature, and transport history for all meals consumed in the field. The time of storage should be evaluated in relation to safety recommendations. An outbreak of disease caused by *Staphylococcus aureus* in a reserve unit, which was caused by ham that had been stored for more than 4 hours in mermite transport containers, is a typical example (JNL, unpublished data, 1993). Investigators must obtain samples from all points of the field water distribution system: the supply pipe line, water trailers, water buffaloes, and individual canteens. They must also document in detail the exact decontamination, disinfection, and chlorination procedures used for the water. Then they must assess the availability of soaps, brushes, and handwashing sites for personal hygiene. It is also critical to review trash management and waste disposal practices and note any unusual aspects of the location altitude and topography. The distance should be measured between field kitchens, water sources, sanitation centers, laundry lines, soakage pits, and garbage pits.

Depending on the outcome of the environmental evaluation, additional clinical specimens may be required for testing, for ova and parasite examinations, or for heavy metals or toxin analysis. Environmental samples must be obtained, transported, and labeled properly. If airborne transmission is of concern, measurement of ventilation rates, adequacy of fresh make up air, and space per occupant should be considered. Air tracer studies with smoke or oil of wintergreen may be useful. Presence of fungal growth could be significant. The limitations of environmental testing methods by sensitivity and specificity, as well as reliability, need to be noted.

An interview and clinical evaluation of workers is frequently a key part of the environmental evaluation of foodborne or waterborne outbreaks, nosocomial epidemics, or other occupational cluster outbreaks. Food handlers, health care staff, or other workers may be case victims as well as vectors. History of recent illnesses with symptoms comparable to the outbreak cases should be carefully obtained because workers are often the earliest cases and may or may not be vectors. Investigators should examine workers for skin lesions on the hands, arms, face, and neck and evaluate them for infection of the respiratory or gastrointestinal tract. Appropriate laboratory specimens, as determined by the presumptive diagnosis, should be obtained from the nasopharynx, throat, hands, or rectum of workers. Interviews should determine in detail the chronological handling of the food, ice, medicine, and other pertinent materials from time of entry until exit from the facility. Food handlers should also be queried concerning their consumption of food and drink.

Team members must identify any potentially critical events occurring in the environment before and during the outbreak. Examples include picnics, parties, sewage spills, field training exercises, construction, floods and heavy rains, and by-passes of the water filtration system. Obtaining detailed information on seemingly irrelevant details, such as recent replacement of pipes in sewage lines or pressure changes due to testing of boilers, may provide the crucial details for unraveling the mechanism of transmission. Six years of drought followed by unusually heavy rains and snows in the spring of 1993 are thought to have contributed to an abundant food supply for the deer-mouse that was ultimately linked to the recognition of hantavirus pulmonary syndrome in North America.[37]

IDENTIFY RISK FACTORS AND MECHANISMS

Frequency Measures and Severity Assessment

After completion of initial assessments, a few cases can be described clinically, incorporating quantitative details obtained by interviewers characterizing symptoms. Organizing frequency of signs and symptoms by percentage of cases may help clarify the disease causing the outbreak. The clinical picture, laboratory results, or estimate of the incubation period from the epidemic curve should help to identify the disease. See Tables 32-1 through 32-3 for a chart of etiological agents in foodborne diseases with their known incubation periods and their associated clinical syndromes. Diagnosis of the disease, number of symptomatic cases, and laboratory results help investigators estimate the symptomatic-to-asymptomatic ratio. Even asymptomatic cases are relevant because they help accurately discriminate cases from susceptible noncases, thus assisting in the rapid development of a tentative hypothesis. Epidemiologists use morbidity measures, such as incidence, prevalence, and attack rates, to quantitatively

TABLE 32-1

CDC GUIDELINES FOR CONFIRMATION OF FOODBORNE DISEASE OUTBREAKS: BACTERIAL

Etiological Agent	Incubation Period	Clinical Syndrome	Confirmation
1. *Bacillus cerus*			
a. Vomiting toxin	1-6 h	Vomiting, some patients withdiarrhea; fever uncommon	Isolation of organism from stool of two or more ill persons and not from stool of controls OR Isolation of ≥10^5 organisms/g from epidemiologically implicated food, provided specimen properly handled
b. Diarrheal toxin	6-24 h	Diarrhea, abdominal cramps, and vomiting in some patients; fever uncommon	Isolation of organism from stool of two or more ill persons and not from stool of controls OR Isolation of ≥10^5 organisms/g from epidemiologically implicated food, provided specimen properly handled
2. *Brucella*	Several days to several mo, usually >30 d	Weakness, fever, headache, sweats, chills, arthralgia, weight loss, splenomegaly	Two or more ill persons and isolation of organism in culture of blood or bone marrow, greater than 4-fold increase in SAT over several wk, or single SAT titer ≥1:160 in person who has compatible clinical symptoms and history of exposure
3. *Campylobacter*	2-10 d, usually 2-5 d	Diarrhea (often bloody), abdominal pain, fever	Isolation of organism from clinical specimens from two or more ill persons OR Isolation of organism from epidemiologically implicated food
4. *Clostridium botulinum*	2 h-8 d, usually 12-48 h	Illness of variable severity; common symptoms are diplopia, blurred vision, and bulbar weakness; paralysis, which is usually descending and bilateral, may progress rapidly	Detection of botulinal toxin in serum, stool, gastric contents, or implicated food OR Isolation of organism from stool or intestine
5. *Clostridium perfringens*	6-24 h	Diarrhea, abdominal cramps; vomiting and fever are uncommon	Isolation of ≥10^6 organisms/g in stool of two or more ill persons, provided specimen properly handled OR Demonstration of enterotoxin in the stool of two or more ill persons OR Isolation of ≥10^5 organisms/g from epidemiologically implicated food, provided specimen properly handled
6. *Escherichia coli*			
a. Enterohemorrhagic (*E coli* O157:H7 and others)	1-10 d, usually 3-4 d	Diarrhea (often bloody), abdominal cramps (often severe), little or no fever	Isolation of *E coli* O157:H7 or other Shiga-like toxin-producing *E coli* from clinical specimen of two or more ill persons OR Isolation of *E coli* O157 or other Shiga-like toxin-producing *E coli* from epidemiologically implicated food
b. Enterotoxigenic (ETEC)	6-48 h	Diarrhea, abdominal cramps, nausea vomiting and fever are less common	Isolation of organisms of same serotype, which are demonstrated to produce heat-stable and/or heat-labile enterotoxin, from stool of two or more ill persons
c. Enteropathogenic (EPEC)	Variable	Diarrhea, fever, abdominal cramps	Isolation of same enteropathogenic serotypefrom stool of two or more ill persons
d. Enteroinvasive (EIEC)	Variable	Diarrhea (may be bloody), fever, abdominal cramps	Isolation of same enteroinvasive serotype from stool of two or more ill persons

7. *Listeria monocytogenes*			
a. Invasive disease	2-6 wk	Meningitis, neonatal sepsis, fever	Isolation of organism from normally sterile site
b. Diarrheal disease	Unknown	Diarrhea, abdominal cramps, fever	Isolation of organism of same serotype from stool of two or more ill persons exposed to food that is epidemiologically implicated or from which organism of same serotype has been isolated
8. Nontyphoidal *Salmonella*	6 h-10 d, usually 6-48 h	Diarrhea, often with fever and abdominal cramps	Isolation of organism of same serotype from clinical specimens from two or more ill persons OR Isolation of organism from epidemiologically implicated food
9. *Salmonella typhi*	3-60 d, usually 7-14 d	Fever, anorexia, malaise, headache, and myalgia; sometimes diarrhea or constipation	Isolation of organism from clinical specimens of two or more ill persons OR Isolation of organism from epidemiologically implicated food
10. *Shigella*	12 h-6 d, usually 2-4 d	Diarrhea (often bloody), frequently accompanied by fever and abdominal cramps	Isolation of organism of same serotype from clinical specimens from two or more ill persons OR Isolation of organism from epidemiologically implicated food
11. *Staphylococcus aureus*	30 min- 8 h, usually 2-4 h	Vomiting, diarrhea	Isolation of organism of same phage type from stool or vomitus of two or more ill persons OR Detection of enterotoxin in epidemiologically implicated food OR Isolation of $\geq 10^5$ organisms/g from epidemiologically implicated food, provided specimen properly handled
12.*Streptococcus* Group A	1-4 d	Fever, pharyngitis, scarlet fever, upper respiratory infection	Isolation of organism of same M- or T-type from throats of two or more ill persons OR Isolation of organism of same M- or T-type from epidemiologically implicated food
13. *Vibrio cholerae*			
a. O1 or O139	1-5 d	Watery diarrhea, often accompanied by vomiting	Isolation of toxigenic organism from stool or vomitus of two or more ill persons OR Significant rise in vibriocidal, bacterial-agglutinating, or antitoxin antibodies in acute-phase and early convalescent–phase sera among persons not recently immunized OR Isolation of toxigenic organism from epidemiologically implicated food
b. non-O1 and non-O139	1-5 d	Watery diarrhea	Isolation of organism of same serotype from stool of two or more ill persons
14. *Vibrio parahaemolyticus*	4-30 h	Diarrhea	Isolation of Kanagawa-positive organism from stool of two or more ill persons OR Isolation of $\geq 10^5$ Kanagawa-positive organisms/g from epidemiologically implicated food, provided specimen properly handled
15. *Yersinia enterocolitica*	1-10 d, usually 4-6 d	Diarrhea, abdominal pain (often severe)	Isolation of organism from clinical specimen of two or more ill persons OR Isolation of pathogenic strain or organism from epidemiologically implicated food

TABLE 32-2

CDC GUIDELINES FOR CONFIRMATION OF FOODBORNE DISEASE OUTBREAKS: CHEMICAL

Etiological Agent	Incubation Period	Clinical Syndrome	Confirmation
1. Marine toxins			
a. Ciguatoxin	1-48 h, usually 2-8 h	Usually gastrointestinal symptoms followed by neurologic symptoms (eg, paresthesia of lips, tongue, throat, or extremities) and reversal of hot and cold sensation	Demonstration of ciguatoxin in epidemiologically implicated fish OR Clinical syndrome among persons who have eaten a type of fish previously associated with ciguatera fish poisoning (eg, snapper, grouper, barracuda)
b. Scombroid toxin (histamine)	1 min-1 h, usually <1 h	Flushing, dizziness, burning of mouth and throat, headache, gastrointestinal symptoms, urticaria, and generalized pruritus	Demonstration of histamine in epidemiologically implicated food OR Clinical syndrome among persons who have eaten type of fish previously associated with histamine fish poisoning (eg, mahi-mahi or fish of order Scomboidei)
c. Paralytic or neurotoxic shellfish poison	30 min-3 h	Paresthesia of lips, mouth or face, and extremities; intestinal symptoms; generalized weakness; respiratory difficulty	Detection of toxin in epidemiologically implicated food OR Detection of large numbers of shellfish-poisoning–associated species of dinoflagellates in water from which epidemiologically implicated mollusks are gathered
d. Puffer fish, tetrodotoxin	10 min-3 h, usually 10-45 min	Paresthesia of lips, tongue, face, or extremities, often following numbness; loss of proprioception or "floating" sensations	Demonstration of tetrodotoxin in epidemiologically implicated fish OR Clinical syndrome among persons who have eaten puffer fish
2. Heavy metals (antimony, cadmium, copper, iron, tin, zinc)	5 min-8 h, usually <1 h	Vomiting, often metallic taste	Demonstration of high concentration of metal in epidemiologically implicated food
3. Monosodium glutamate (MSG)	3 min-2 h, usually <1 h	Burning sensation in chest, neck, abdomen, or extremities; sensation of lightness and pressure over face or heavy feeling in chest	Clinical syndrome among persons who have eaten food containing MSG (ie, usually ≥1.5 g MSG)
4. Mushroom toxins			
a. Shorter-acting toxins:	≤2 h	Usually vomiting and diarrhea, other symptoms differ with toxin:	Clinical syndrome among persons who have eaten mushroom identified as toxic type OR Demonstration of toxin in epidemiologically implicated mushroom or mushroom-containing food
Muscimol		Confusion, visual disturbance	
Muscarine		Salivation, diaphoresis	
Psilocybin		Hallucinations	
Coprinus artrementaris		Disulfiram-like reaction	
Ibotenic acid		Confusion, visual disturbance	
b. Longer-acting toxin (eg, *Amanita* spp)	6-24 h	Diarrhea and abdominal cramps for 24 h followed by hepatic and renal failure	Clinical syndrome among persons who have eaten mushroom identified as toxic type OR Demonstration of toxin in epidemiologically implicated mushroom or mushroom-containing food

TABLE 32-3

CDC GUIDELINES FOR CONFIRMATION OF FOODBORNE DISEASE OUTBREAKS: PARASITIC AND VIRAL

Etiological Agent	Incubation Period	Clinical Syndrome	Confirmation
Parasitic			
1. *Cryptosporidium parvum*	2-28 d, median: 7 d	Diarrhea, nausea, vomiting, fever	Demonstration of organism or antigen in stool or in small-bowel biopsy of two or more ill persons OR Demonstration of organism in epidemiologically implicated food
2. *Cyclospora cayetanensus*	1-11 d, median: 7 d	Fatigue, protracted diarrhea, often relapsing	Demonstration of organism in stool of two or more ill persons
3. *Giardia lamblia*	3-25 d, median: 7 d	Diarrhea, gas, cramps, nausea, fatigue	Two or more ill persons and detection of antigen in stool; or demonstration of organism in stool, duodenal contents, or small-bowel biopsy specimen
4. *Trichinelle* spp	1-2 d for intestinal phase; 2-4 wk for systemic phase	Fever, myalgia, periorbital edema, high eosinophil count	Two or more ill persons and positive serologic test or demonstration of larvae in muscle biopsy OR Demonstration of larvae in epidemiologically implicated meat
Viral			
1. Hepatitis A	15-50 d, median: 28 d	Jaundice, dark urine, fatigue, anorexia, nausea	Detection of IgM anti-hepatitis A virus in serum from two or more persons who consumed epidemiologically implicated food
2. Norwalk family of viruses (SRSVs)	15-77 h, usually 24-48 h	Vomiting, cramps, diarrhea, headache	More than 4-fold rise in antibody titer to Norwalk virus or Norwalk-like virus in acute and convalescent sera in most serum pairs OR Visualization of SRSVs that react with patient's convalescent sera but not acute sera—by immune-electron microscopy; assays based on molecular diagnostic (eg, polymerase chain reaction, probes, assays for antigen and antibodies from expressed antigen) are available in reference laboratories
3. Astrovirus, calicivirus, others	15-77 h, usually 24-48 h	Vomiting, cramps, diarrhea, headache	Visualization of SRSVs that react with patient's convalescent sera but not acute sera—by immune-electron microscopy; assays based on molecular diagnostics (eg, PCR, probes, or assays for antigen and antibodies from expressed antigen) are available in reference laboratories

SAT: standard agglutination titer
SRSV: small round-structured viruses
Source: Centers for Disease Control and Prevention. Guidelines for confirmation of bloodborne-disease outbreaks. *MMWR*. 1996;45(SS-5):59–66.

describe disease or injury among a population. Attack rates by sex, military unit, residence, or other factors may provide helpful clues. Ideally all members of a denominator should be eligible to enter the numerator in a rate calculation. For example, only persons susceptible to hepatitis A should be in the denominator of a hepatitis A attack rate. The investigators must calculate secondary attack rates in unit members, family members, or contacts of ill service members who had no exposure to the presumed primary event or source. Attack rates by history of food and drink consumption play a key role in the investigation of foodborne and waterborne outbreaks.

Severity can be assessed by calculating the days lost from training, work, or duty as disability days or by the duration of hospitalization. In severe disease or injury clusters, case fatality rates and mortality rates will also be used and should be compared with those in the literature for the specified condition. In all rates, the definition of a case and the limitations of the data source must be specified. This is especially critical when using medical records to identify quantitative measures.

Epidemiologic Measures of Risk

To establish risk factors, a variety of epidemiologic measures of risk are available. Either chi-square or Fisher's exact test is used to test the association between categorical variables. Relative risk calculated for a cohort is the critical measure for assessing the etiological role of a factor in a disease. The relative risk reflects the excess risk in the exposed group when compared with the unexposed group. The risk or attack rate in an acute outbreak setting in the exposed group is divided by the risk or attack rate in the unexposed group. Case-control studies are often used in outbreak investigations as a means to identify significant risk factors. For case-control studies, the excess risk cannot be measured directly because the exact denominator population (needed to calculate attack rates) is not known. The odds ratio is the most commonly used measure of risk for case-control studies. The odds ratio calculated from a standard 2 x 2 table is ad/bc and from a matched case-control study is b/c. Fisher's exact test is used when any of the expected values for a 2 x 2 contingency table is less than five.[38] Chi-square or Fisher's exact test can be used to assess the significance of observed effects against the null hypothesis. Confidence intervals can also be calculated for relative risk or odds ratios to determine if the measure includes or excludes 1.0.

Attributable risk examines the contribution of an exposure to the frequency of a disease in a population. In an epidemic, it is expected that most of the cases become ill because of an exposure to the imputed risk factor. The population attributable risk percentage represents the proportion of disease in a population attributable to an exposure. It reflects both the relative risk and the frequency of the factor in the population. It can be calculated by subtracting the risk in the unexposed from the risk in the exposed and then dividing by the risk in the exposed. This fraction, called the etiologic fraction, would then be multiplied by 100 and reported as a percentage.

DEVELOP TENTATIVE HYPOTHESES AND A PLAN

The information gathered to this point by the investigation team should be sufficient to allow them to formulate a tentative hypothesis and further refine the investigation plan.

Determine Type of Outbreak and Critical Exposure

The type of outbreak—point or continuing common source, person-to-person propagation, or mixed—should be evident from the epidemic curve. Initial case interviews and the environmental investigation may have identified a presumed critical exposure at an event or from a particular source. Synthesizing facts on the epidemiology and clinical characteristics of a disease, host factors, role of vectors, and importance of reservoirs should lead to a presumed mechanism of transmission. A tentative theory (or theories) that explains the observed pattern of disease in a given environmental situation should have surfaced. Initially several broad hypotheses may be under consideration.

Hypothesize About Mechanism of Transmission

The complexity of our global community will make identification of risk factors and mechanisms of transmission extremely difficult. The shifting epidemiology of foodborne diseases in the United States during recent years caused by changes in food production and distribution methods warrants additional discussion.[39] The traceback of an *Escherichia coli* O157H outbreak implicating meat is an example. The traceback might take investigators from a dining facility to a distribution center to a meat processing plant to a boning and packaging plant to a slaughter plant to feed lot auctions to individual

ranchers.[40] Along the way, meats from multiple sources are mixed, and contamination could occur at many points in this complex chain. The largest (224,000 cases) common-vehicle outbreak of salmonellosis ever recognized in the United States implicated a nationally distributed brand of ice cream by a company that provided home delivery. Investigators found that cross-contamination of pasteurized ice cream premix occurred during transport in tanker trailers that had previously hauled nonpasteurized liquid eggs containing *Salmonella enteritidis*.[41] Extensive tracebacks to identify the source of a pathogen are most useful when the implicated vehicle is either novel or has a long shelf life. The increase in foreign travel and the internationalization of the market for food supplies and other commerce also vastly complicates investigations.[42] Implicated in a 1997 outbreak of hepatitis A were contaminated strawberries imported from Mexico that were distributed to at least six states.[43] Military investigators must be prepared to deal with multinational outbreaks and should not be surprised to identify novel vehicles, new mechanisms, or an unusual chain of events contributing to the occurrence of outbreaks.

The possibility of sabotage or purposeful contamination should always be considered. A single act of terrorism that contaminates a water supply can place an entire force at risk. Identification of unusual diseases or rare strains may be the first indication of an unnatural event. A gastroenteritis outbreak of *Shigella dysenteriae* type 2, which is rare in the United States, occurred in 1996 following the removal of a stock culture of the organism from a medical center's laboratory. Health care workers at the medical center became ill after eating food that had been maliciously contaminated.[44] If the terrorists do not make demands or claim responsibility, as was the case in this outbreak, it may be extremely difficult to recognize that the contamination of food or water did not occur naturally. Another example of sabotage that was initially unrecognized was the largest foodborne outbreak reported in the United States in 1984.[45] Evidence obtained in an independent criminal investigation was essential to determining that members of a religious commune had intentionally contaminated restaurant salad bars with *Salmonella typhimurium*. Good laboratory work helped demonstrate that the *Salmonella* type was one found in a reference type collection rather than a strain more typically found in general circulation. This outbreak also demonstrates the vulnerability of self service foods to intentional contamination. Although recognition of an outbreak caused by a biological warfare attack can be quite challenging, a number of indicators listed in Exhibit 32-4 should

EXHIBIT 32-4

INDICATIONS OF POSSIBLE BIOLOGICAL WARFARE ATTACK

A disease entity (sometimes even a single case) that is unusual or that does not occur naturally in a given geographic area, or combinations of unusual disease entities in the same patient population
Multiple disease entities in the same patients, indicating that mixed agents have been used in the attack
Large numbers of both military and civilian casualties when such populations inhabit the same area
Data suggesting a massive point-source outbreak
Apparent aerosol route of infection
High morbidity and mortality relative to the number of personnel at risk
Illness limited to fairly localized or circumscribed geographical areas
Low attack rates in personnel who work in areas with filtered air supplies or closed ventilation systems
Sentinel dead animals of multiple species
Absence of competent natural vector in the area of outbreak (for a biological agent that is vector-borne in nature)

Source: Wiener SL, Barratt J. Biological warfare defense. In: *Trauma Management for Civilian and Military Physicians*. Philadelphia: WB Saunders; 1986.

suggest the possibility.[46] The disease pattern is an important factor in differentiating between a naturally occurring outbreak and a terrorist attack.[47] Terrorist objectives may include inducing a large number of cases, so health care workers may see many cases presenting simultaneously. This compressed epidemic curve with a very high case-to-exposure rate contrasts with the more gradual rise in disease incidence expected in most naturally occurring epidemics. Animals may also be affected by biological or chemical warfare attacks. Disease may appear in unexpected geographic areas that lack the normal vector for transmission. Unusual clinical presentations may occur because of a combination of agents or altered routes of transmission induced by the saboteur. The accidental release of aerosolized anthrax from a Russian biological weapons facility in 1979 resulted in respiratory instead of cutaneous disease, and the location of cases followed a distinctive downwind pattern from the site of release.[48] Anthrax continues to be considered as a biological warfare agent. The Aum Shinrikyo cult members arrested following the sarin attack in a Tokyo subway were also conducting research on anthrax and botulinum toxin.[12] Drone aircraft equipped with spray tanks found in the cult's arsenal made the potential for aerosolization of these agents a real threat. Some terrorist actions may be recognized only when first responders become secondary cases from toxic gas exposure. Thirteen of fifteen emergency room doctors treating victims of the sarin attack in Japan noted onset of their own symptoms while they were resuscitating victims.[49] Simultaneous outbreaks of multiple agents should also raise suspicion of biological terrorist etiology.

Identification of risk factors for the formulated hypothesis should determine the need for more specialized tests or for outside expert consultation in the appropriate field, be it medicine, engineering, entomology, or other fields. The differential diagnosis should be further narrowed, and the collection of the optimal source and type of specimens should be started, if not already underway. See chapter 34 for collection, transport, and processing considerations. Laboratory and field instruments must be calibrated. The specific laboratory designated to support the investigation should be fully aware of the tentative hypotheses of the team.

Develop Survey Instruments

Investigators should incorporate standard methods for designing questionnaires used for detailed risk factor interviews.[50] The instrument must achieve the specific objectives of the investigation. Items should be simple and unambiguous. Included should be exposure information and demographic factors, as well as clinical history and host factors that may affect risk. Any potentially relevant history, including such factors as chronic disease, nutrition, housing conditions, crowding, work locations, job, stress, pets, source of food, and source of water, should be obtained. The possibility of exotic house pets (eg, iguanas, snakes, hedgehogs, ferrets) or stray animals adopted in the field may be important. A site visit and some preliminary interviewing in the early designing of the questionnaire will improve the sensitivity and specificity of the instrument. Leading questions and lengthy surveys should be avoided. Survey design must take into account the coding scheme for entering the data into a database and whether data entry will occur in the field as it is collected or at a later time. Forms that can be optically scanned may be useful to facilitate data entry. Investigators must consider the data analysis methods planned to accomplish specific study objectives and construct empty tables for variables of interest in which data can be inserted after collection. Validation and quality control checks of the data should be planned. On-site scanning equipment can help with this. Figure 32-3 is a questionnaire designed by the CDC for use in a foodborne disease outbreak.

Cases and controls from the population under study should be interviewed in a consistent fashion using the survey tools developed. Interviewers must strive to create a nonthreatening environment in which those being questioned feel free to share all possible information without punishment. Initial questions should be simple and designed to put the subject at ease. For large, complex, or unusual outbreaks, interviewers should be trained first in both interviewing technique and the subject matter under investigation.[51] Time used to pretest the proposed questionnaire adequately is time well spent because pretesting can identify unclear or problem questions. The questionnaire and plans for its administration should be revised in accordance with the results of the pilot testing. As a quality control measure, each survey should be reviewed for legibility and completeness as it is returned. The same principles of developing forms, training interviewers, and pretesting forms and procedures apply to the process of abstracting data from medical records.

Most field outbreak investigations are the "emergencies" of the specialty of preventive medicine. Thus the time-sensitive investigation of this acute public health problem is considered operational and

CDC USE ONLY ☐☐☐☐ (1-4)

This report is authorized by law (Public Health Act, 42 USC 241). While your response is voluntary, your cooperation is necessary for the understanding and control of the disease.

FORM APPROVED
OMB NO. 0920-0004

INVESTIGATION OF A FOODBORNE OUTBREAK

1. Where did the outbreak occur ?

State _____ (5-6) City or Town _____ County _____

2. Date of outbreak: (Date of onset 1st case)

_____
MO / DA / YR (7-12)

3. Indicate actual(a) or estimated (e) numbers:

Persons exposed _____ (13-17)

Persons ill _____ (18-22)

Hospitalized _____ (23-27)

Fatal case _____ (28-31)

4. History of Exposed Persons;

No. histories obtained _____ (32-35)

No. persons with symptoms _____ (36-39)

Nausea _____ (40-43) Diarrhea _____ (44-47)

Vomiting _____ (48-51) Fever _____ (52-55)

Cramps _____ (56-59) Other, specify _____

_____ (60-79)

5. Incubation period (hours):

Shortest _____ (80-83) Longest _____ (84-87)

Approx. for majority _____ (88-91)

6. Duration of illness (hours):

Shortest _____ (92-95) Longest _____ (96-99)

Approx. for majority _____ (101-104)

7. Food - specific attack rates:

Food Items Served	Number of persons who ATE specified food				Number who did NOT eat specified food			
	Ill	Not Ill	Total	Percent Ill	Ill	Not Ill	Total	Percent Ill

8. Vehicle responsible (food item incriminated by epidemiological evidence): (105-106) _____

9. Manner in which incriminated food was marketed: (Check all Applicable)

	Yes 1	No 2		Yes 1	No 2	
(a) Food Industry			(c) Not Wrapped	☐	☐	(112)
Raw	☐	☐ (107)	Ordinary Wrapping	☐	☐	(113)
Processed	☐	☐ (108)	Canned	☐	☐	(114)
Home Produced			Canned—Vacuum Sealed	☐	☐	(115)
Raw	☐	☐ (109)	Other (specify)	☐	☐	(116)
Processed	☐	☐ (110)	_____			(117-129)
(b) Vending Machine	☐	☐ (111)	(d) Room Temperature	☐	☐	(130)
			Refigerator	☐	☐	(131)
			Frozen	☐	☐	(132)
			Heated	☐	☐	(133)

If a commerical product, indicate brand name and lot number

_____ (134-150)

10. Place of Preparation of Contaminated Item: (151)

Restaurant ☐ 1
Delicatessen ☐ 2
Cafeteria ☐ 3
Private Home ☐ 4
Caterer ☐ 5
Institution:
School ☐ 6
Church ☐ 7
Camp ☐ 8
Other, specify ☐ 9

_____ (152-171)

11. Place where eaten: (172)

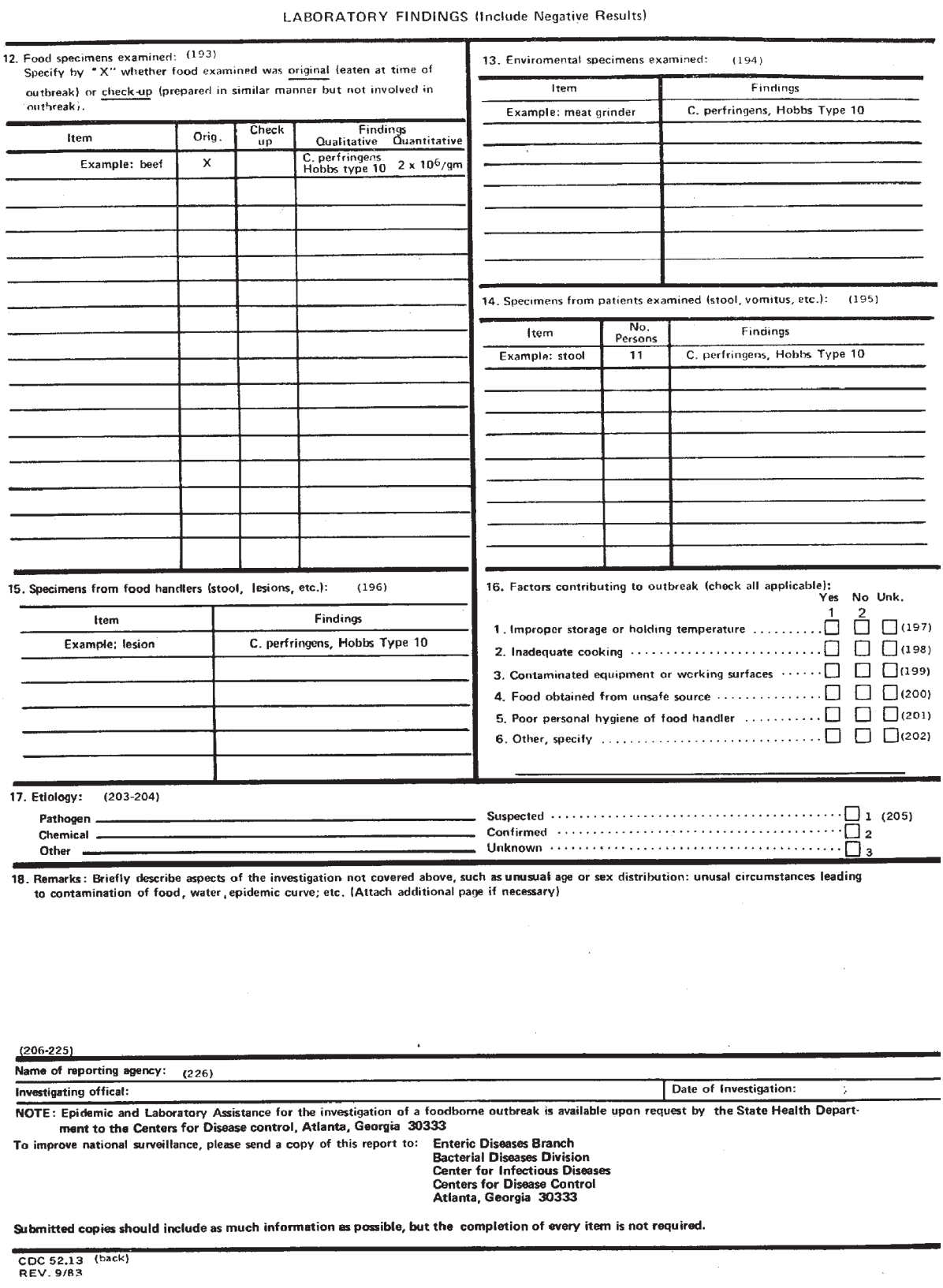
Restaurant ☐ 1
Delicatessen ☐ 2
Cafeteria ☐ 3
Private Home ☐ 4
Picnic ☐ 5
Institution:
School ☐ 6
Church ☐ 7
Camp ☐ 8
Other, specify ☐ 9

_____ (173-192)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
CENTERS FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333

CDC 52.13 REV. 9/83 (over)

(**Fig.32-3** *continues*)



LABORATORY FINDINGS (Include Negative Results)

12. Food specimens examined: (193)
Specify by "X" whether food examined was original (eaten at time of outbreak) or check-up (prepared in similar manner but not involved in outbreak).

Item	Orig.	Check up	Findings Qualitative	Findings Quantitative
Example: beef	X		C. perfringens Hobbs type 10	2×10^6/gm

13. Enviromental specimens examined: (194)

Item	Findings
Example: meat grinder	C. perfringens, Hobbs Type 10

14. Specimens from patients examined (stool, vomitus, etc.): (195)

Item	No. Persons	Findings
Example: stool	11	C. perfringens, Hobbs Type 10

15. Specimens from food handlers (stool, lesions, etc.): (196)

Item	Findings
Example: lesion	C. perfringens, Hobbs Type 10

16. Factors contributing to outbreak (check all applicable):

	Yes 1	No 2	Unk.	
1. Improper storage or holding temperature	☐	☐	☐	(197)
2. Inadequate cooking	☐	☐	☐	(198)
3. Contaminated equipment or working surfaces	☐	☐	☐	(199)
4. Food obtained from unsafe source	☐	☐	☐	(200)
5. Poor personal hygiene of food handler	☐	☐	☐	(201)
6. Other, specify	☐	☐	☐	(202)

17. Etiology: (203-204)

Pathogen _____
Chemical _____
Other _____

Suspected ☐ 1 (205)
Confirmed ☐ 2
Unknown ☐ 3

18. Remarks: Briefly describe aspects of the investigation not covered above, such as unusual age or sex distribution: unusal circumstances leading to contamination of food, water, epidemic curve; etc. (Attach additional page if necessary)

(206-225)

Name of reporting agency: (226)

Investigating offical: Date of Investigation:

NOTE: Epidemic and Laboratory Assistance for the investigation of a foodborne outbreak is available upon request by the State Health Department to the Centers for Disease control, Atlanta, Georgia 30333

To improve national surveillance, please send a copy of this report to:
Enteric Diseases Branch
Bacterial Diseases Division
Center for Infectious Diseases
Centers for Disease Control
Atlanta, Georgia 30333

Submitted copies should include as much information as possible, but the completion of every item is not required.

CDC 52.13 (back)
REV. 9/83

Fig. 32-3. This is an example of a questionnaire developed by the Centers for Disease Control and Prevention to be used in foodborne disease outbreaks.
Source: Reference: Centers for Disease Control and Prevention. Guidelines for confirmation of bloodborne-disease outbreaks: appendix A. *MMWR*. 1996;45(SS-5):56–57.

does not require a preapproved protocol in most circumstances. However, human experimental testing consisting of invasive procedures or procedures having risks greater than that encountered in daily life would require a research protocol approved by an Institutional Review Board.[52] Investigations using surveys containing questions on sensitive subjects (eg, sexual history, drug or alcohol consumption, illegal activities) also are subject to the federal policy for protection of human subjects and may require voluntary informed consent. Title 45 of the Code of Federal Regulations Part 46 Subpart A-D establishes Institutional Review Boards as the approval authority for research conducted by federal agencies or other institutions conducting research supported by federal funds. Current military regulations and service clinical investigation consultants should be consulted to ensure compliance with both regulatory and ethical standards.

Plan for Administrative and Logistical Considerations

Following the initial in-brief, the investigation team needs to provide the local chain of command with regular updates on the progress of the investigation. A local command liaison should attend all team meetings and can expedite obtaining additional supplies or other administrative support. This should obviate the need for daily meetings of the team with the local commander, who will be kept informed by his or her liaison. Periodic meetings with the commander can then occur as dictated by progress in the investigation. The team leader will determine the specific type and magnitude of additional help needed based on the level of expertise available locally. The specific number of personnel needed by discipline (eg, lab technician, data entry clerk, nurse interviewers) should be assessed. Resources can include both military and civilian public health officials. The Army has the Epidemiologic Consultant Service (EPICON), the US Public Health Service has investigation teams from the CDC using Epidemic Intelligence Service officers, and states have various capabilities within state and local health departments. Reporting of diseases to military health authorities and to state health departments as required by law must also not be forgotten.

The team leader must establish clear operational priorities and then ensure a systematic and orderly progress of the investigation in all areas: clinical, laboratory, environmental, and epidemiologic. Tracking the status and progress of the simultaneous actions taking place is one of the biggest challenges for the leader. Logging all decisions and delegating specific taskings at team meetings are tools the leader can use to help keep the investigation team on track. The team cannot afford the time to follow each phase of the investigation sequentially to completion before beginning to pursue knowledge in another area. The purpose of the multidisciplinary consultant team is to allow each expert to concentrate on those factors within his or her discipline that may have contributed to the event under investigation. Reference materials and experts should be consulted as new facts emerge. Working as a team adds the necessary intellectual synergy to the complex investigation process. At each team meeting, investigators assimilate new data resulting from the efforts of their colleagues. The ensuing discussion should result in productive, thoughtful analysis. But many decisions will still have to be made with inconclusive or inadequate data. Laboratory analysis is frequently still incomplete when critical decisions have to be made. The team leader's fund of clinical and epidemiologic knowledge and his or her experience in making judgement calls in these high-pressure public health emergencies will be of the utmost value. The team leader must continually synthesize new data as they accrue, keep all phases on track, and direct the future lines of inquiry of the investigation. The team leader should seek advice from the off-site consultant on a daily basis, but the team leader on-site must have ultimate decision-making authority for the team.

Suspicion of a biological warfare attack mandates additional immediate responses: reporting to local military police and to the Federal Bureau of Investigation.[47] Rapidity of communicating this suspicion up the military chain of command is especially critical in a theater of operation. The Federal Bureau of Investigation is responsible for crisis management, which includes actions taken before an incident to avert it. The Federal Emergency Management Agency is in charge of consequence management or actions taken after the incident to mitigate its effects.[53] Department of Defense staff are key players in both phases of operations precipitated by such incidents. Because terrorism generates panic in a population, coordination to establish an emergency operations center and to enhance security must be one of the initial steps taken. Ongoing communication between law enforcement officials and health authorities is critical to the optimal investigation and management of such incidents. Rapid transport of specimens to the US Army Research Institute of Infectious Diseases, US Navy Medical Research Center, and the CDC is vital to obtain an accurate, rapid diagnosis where biological attack is a consideration.

Identify Medical Treatment Resources

Field triage sites, outpatient clinics, emergency rooms, and hospitals that can receive and treat potential cases should be identified and a list of them provided to the team public affairs officer for distribution to the media. This will allow the investigation team to focus on conducting the investigation and making appropriate prevention and control recommendations. Clinicians should be notified when to expect cases and briefed on presumed condition, appropriate diagnostic evaluation, treatment regimens, and any recommended isolation precautions. Education of medical staff may be needed to update them on rare conditions or to explain occupational risks and precautions warranted. Guidelines for transfer or evacuation of patients to the next echelon of care should be established and the referral center identified. If the saboteur used a biological agent, there will probably be an immediate clamor for large quantities of medical supplies, such as antibiotics or vaccines, for which only limited quantities exist.[12] The priority of use and specific distribution plans for these limited resources should be addressed.

Reevaluate Controls Measures in Place

This periodic reassessment of preventive medicine controls should include degree of compliance and adequacy of the initial guidelines in view of the latest information. The team must determine the need for additional strategies and resources, whether personnel or supplies. The need to open an additional unit or an entire field hospital should be assessed. The need for any product recall should also be assessed. The initiation and timing of such actions require considerable judgement to weigh the preponderance of the evidence, the strength of the hypothesized association, and the severity of the risk to individuals if a recall is delayed. Such decisions may have great economic impact on an industry. Epidemiologists must balance the need to warn the public against the damage of falsely accusing an industry or other postulated source. Unfounded allegations could result in unnecessary economic losses, such as occurred to the California strawberry industry during the cyclosporiasis outbreaks.[54] Mistakes in identifying etiological agents may also lead to the loss of public confidence in future public health warnings.

TEST THE HYPOTHESIS

At this point in the investigation, investigators have postulated risk factors and developed a hypothesis that explains the source, mode of transmission, and duration of the epidemic. Hypotheses are now tested by determining whether or not a statistical association exists between two categories: exposure (eg, to a specific food or chemical) and clinical outcome (eg, illness or injury). The analysis of the results collected to date and the statistical testing of these results will determine the accuracy of the hypothesis specifying a risk factor to be the cause of the epidemic.

Collect Data and Specimens

Follow-up of the clues provided from the description of the epidemic by person, time, and place, and laboratory analysis should result in confirmation of the diagnosis. All possible cases should be identified. Both cases and appropriate controls should be interviewed and given the survey tool to obtain data that will establish risk factors. From these data and the environmental evaluation, the mechanisms of transmission are determined. The cross-sectional or case-control data collected must be analyzed. Investigators should calculate attack rates of symptomatic disease from the suspected risk factors. If food is the suspected exposure and a suspected dining facility serves multiple meals, meal-specific attack rates should be calculated first. To do this, investigators must know the number of cases and controls who did and did not eat the specific meals being compared. When a single suspect meal is obvious (eg, a banquet), investigators can initially calculate the food-specific attack rates. Similarly they must determine the number of cases and the number of controls who did and did not eat the specific food item in question. Investigators compare the rates of illness for those who were and were not exposed via consumption of a particular food or drink item by calculating the differences between the disease attack rate for those who did eat the food item and those who did not. The food item that shows the greatest percentage difference is the most likely source. Combining attack rates is often useful (eg, potatoes and gravy, ice cream and chocolate sauce, ice and water). The highest attack rates will be observed in all combinations involving the suspected food. Cumulative food attack rates may be calculated when a specific food is served on more than one occasion to the same population of people. Next is the calculation of the difference between cumu-

TABLE 32-4

***SHIGELLA DYSENTERIAE* TYPE 2 OUTBREAK, CHI SQUARE AND P VALUES FOR COMMON FOOD ITEMS**

	Cases		Controls			
Food	**Ate**	**Did Not Eat**	**Ate**	**Did Not Eat**	**Chi Square**	***p* Value**
Salad	80	9	35	26	21.38	0.0001*
Grilled sandwich	27	62	28	33	3.78	0.0520
Fruit cocktail	11	78	8	53	0.02	0.8913
Ice	56	33	33	28	1.17	0.2799
Water	27	62	12	49	2.14	0.1435
Soft Drink	41	48	30	31	0.14	0.7076
Punch	19	70	9	52	1.04	0.3086
Lemonade	9	80	10	51	1.29	0.2559
Iced tea	6	83	2	59	0.86	0.3538
Milk	52	37	20	41	9.53	0.0020*
Coffee/tea	17	72	15	46	0.65	0.4202
Hot chocolate	4	85	2	59	0.14	0.7090
French fries	31	58	20	41	0.07	0.7951
Ice cream	50	39	24	37	4.10	0.0428*

*p Value < .05

Source: Centers for Disease Control. Hospital-associated outbreak of *Shigella dysenteriae* type 2—Maryland. *MMWR*. 1983;32:250–252.

lative attack rates for each specific food between cases and controls. Then a statistical test is applied to determine if there are significant differences between foods as a risk factor for the illness. Tests used for these purposes include chi-square and Fischer's Exact Test for individual food comparisons as shown in Table 32-4. Other risk measures investigators may apply are odds ratios with confidence intervals for univariate and stratified Mantel-Haenszel analyses. In a case-control design, investigators can study the discordant exposures of cases. Testing a hypothesis using data from a case-control design can be an extremely powerful tool to identify a contaminated vehicle when dealing with mega-outbreaks. A single case-control study of 15 matched pairs provided the evidence implicating the ice cream in the massive salmonellosis outbreak referred to previously. This association was demonstrated 10 days before the isolation of the organism from the ice cream.[41] Control measures were taken based on this statistical association before laboratory confirmation, thus preventing many additional cases.

Because factors other than the etiological risk factor may affect outcome, a stratified analysis is sometimes required. This involves examining the exposure–disease association within different categories of a third factor. The third factor is referred to as the confounder. This is an effective method for looking at the effects of two different exposures on the disease, and it is one way to tease apart association with multiple factors, such as two foods. The most common method used to control for confounding is to stratify the data and then compute measures that represent weighted averages of the stratum-specific data using methods such as the Mantel-Haenszel formula.[55] The Mantel-Haenszel formula was used to analyze the data from the shigella outbreak to differentiate among salad, milk, and ice cream as the etiological agent (Table 32-5).

Stratification is also used to assess effect modification or interaction. Effect modification refers to the situation in which the degree of association between an exposure and an outcome differs in different subgroups of the population. Evaluation for effect modification is accomplished by determining whether the stratum-specific odds ratios differ from one another. There are more complete discussions of analysis strategies for outbreak investigations elsewhere.[56]

TABLE 32-5

***SHIGELLA DYSENTERIAE* AND CONSUMPTION OF SALAD, MILK, AND ICE CREAM**

Rate	Salad	Milk	Ice Cream
Crude odds ratio	6.60	2.89	1.98
MH OR adjusted for salad, 95% (CL)	—	2.29 (1.10-4.77)	1.65 (0.80-3.41)
MH OR adjusted for milk, 95% (CL)	5.87	—	—
MH OR adjusted for ice cream, 95%(CL)	5.99 (2.49-14.42)	—	—

MH: Mantel-Haenszel
OR: odds ratio
CL: confidence limits
Source: Centers for Disease Control. Hospital-associated outbreak of *Shigella dysenteriae* type 2—Maryland. *MMWR*. 1983;32:250–252.

Determine the Need for Additional Studies and Analysis

If the results of the investigation thus far have not yielded a definitive diagnosis and a presumed mechanism of disease transmission, then additional investigation is indicated. A more detailed questionnaire may be needed to elucidate new details. More sensitive or sophisticated laboratory testing or additional environmental consultation may be necessary. Investigators should consider whether the initiation of carriage studies in controls or other populations at risk would be helpful. Successful outbreak investigations will frequently depend on the critical integration of epidemiologic and laboratory sciences. The application of molecular techniques to analyze epidemiologic interrelationships has led to the use of the term molecular epidemiology. Traditional laboratory methods to characterize epidemic strains have relied on the measurement of phenotypes, such as antibiotic resistance, phage typing, or serotyping. Plasmid profile analysis was the first molecular tool to fingerprint bacteria. The newer techniques can potentially type any strain by using the chromosomal DNA present in all bacteria and fungi. Genomic (chromosomal) digests, restriction fragment length polymorphisms (RFLPs), and PFGE analyze differences in chromosomal DNA organization.[57] The PFGE is increasingly being viewed as a new gold standard for the epidemiologic analysis of nosocomial infection,[58] and applications to disease outbreaks caused by bacterial contamination of food will become routine in the future. Molecular biology will often be key to identifying previously unknown infectious disease agents, as in the case of human ehrlichiosis.[59] Molecular biological methods may be used to determine early in the investigation whether a single strain or type of microorganism is responsible for the majority of cases. Linkage to a single strain suggests that patients were exposed to a common source or reservoir. Molecular analysis of dengue viruses was a useful adjunct to the epidemiologic investigation of virus distribution over distance and time in US personnel in Somalia.[60] Molecular techniques can be applied to the study of reservoirs of infection and to trace the modes of transmission.

Epidemiologists must be willing to reject early theories if initial hypothesis testing is not statistically significant. Examination of alternative hypotheses to explain investigation findings is the next step. The process is iterative in nature, as a hypothesis is tested and rejected and then followed by new planning to explore alternative explanations for the event under study.[61] Subsequent studies may involve additional data collection before testing the next hypothesis under consideration. Subsequent iterations of this process should accurately determine the true risk factors or etiology. Initial investigations of three outbreaks of infection with *Cyclospora* attributed the risk to consumption of strawberries at special events. However, this was inconsistent with the observation that while cases occurred primarily in the eastern United States, most strawberries are grown in the western United States. Further investigation showed that raspberries from Guatemala (sometimes served with strawberries) were the actual vehicle.[62] Even if the data collected are sufficient, a more sophisticated analysis method, such as logistic regression, may be required to identify the underlying association.

Evaluate the Adequacy of the Case Definition

Case confirmation by laboratory testing permits evaluation of the sensitivity and specificity of the definition used during the outbreak investigation. Additional systematic studies may attempt to find more cases, obtain more data, (clinical, laboratory, or epidemiologic), or both. Use of serologic data and clinical history may improve the case definition and clarify the population at risk for disease. Repeat interviews of confirmed cases may reveal quantitative data on exposure dose. If multiple working case definitions were used, the sensitivity of each case definition can now be compared (eg, definite versus probable, primary versus secondary).

Establish Criteria for Deciding the Outbreak Is Under Control

Surveillance to find new cases as they occur should be ongoing. The investigators should have established numerical criteria for determining that the outbreak is under control. They must consider whether this is a complete return to preoutbreak levels or whether it is the expected small numbers of cases that will occur as the final contacts of cases in a propagated epidemic pass through the incubation period window. In almost all cases of infectious disease outbreaks and in selected occupational clusters, some type of surveillance system should be established to monitor the situation for a specified period following the outbreak. Ascertaining cases of gastroenteritis appearing at sick call in a field unit following a foodborne epidemic or monitoring wound infections by surgeon or operating room in certain nosocomial outbreaks are typical examples.

To ensure a smooth transition from the consultant team to local health workers, special care should be taken to hand off responsibilities and follow-on investigation taskings (eg, laboratory results follow-up, new surveillance activities). Timing and details of the transition should be clearly delineated before the team departs.

COMPARE RESULTS TO THOSE OF THE PUBLISHED LITERATURE

After the field investigation and the subsequent analysis are complete, it is important to thoughtfully compare the findings with those conducted previously and recorded in the medical literature. Frequently the introduction of new diagnostic methods or technological advances in the interim will have allowed a new understanding of pathophysiological mechanisms that can help explain associations. New insights or the validation of new approaches should be published.

FINALIZE A REPORT WITH RECOMMENDATIONS TO CONTROL FURTHER SPREAD

Write an Executive Summary and Prepare a Complete Report

Updating local health officials and local command authorities on the investigation results is essential before the team's departure. This out-briefing should include diagnosis, risk factors, presumed etiology, treatment, environmental controls, surveillance plan, diagnostic improvements, and additional preventive measures for the future. This preliminary report may be oral but must be followed by a written summary. A final executive report summary written in language appropriate for nonmedical commanders should be provided to the local commanders of the site and unit involved. A more detailed final report should be prepared after all the analyses are completed. This final report should define the problem, describe the methods used in the field investigation, display the epidemiologic analysis, discuss the results, and present the final conclusions and recommendations. It should be submitted to the appropriate medical authorities. This documentation of the epidemic and the investigation findings is important for possible future use to support or justify changes in public health decisions. In certain situations, rapid publication of preliminary results in the *Morbidity and Mortality Weekly Report* or internal organizational publications is important to alert health care providers or health authorities of apparent new risks or diseases. Rapid initial disease report summaries may also serve to alert civilian physicians to potential problems, such as malaria, in redeploying service members.[63]

Evaluate the Effectiveness of Recommendations

An assessment plan to evaluate the effectiveness of control procedures and recommendations should be part of the final report. Specific surveillance for the disease or condition of interest following the departure of the team is critical. Active surveillance is preferred over passive surveillance to detect any subsequent outbreaks, as well as to evaluate the effect of the control measures.

SUMMARY

Epidemiology is the fundamental science of public health, and outbreak investigation is the most visible example of applied field epidemiology. Results from field studies are critical to finding effective interventions for disease control. The great epidemiologic challenge of today is the development of new tools, such as molecular epidemiology, to apply to the investigation of emerging infections. Thoughtful, careful investigation will continue to be necessary to identify new pathogens, find their natural reservoirs, and determine their routes of transmission. This will not be easy. For example,

despite the occurrence of outbreaks of Ebola hemorrhagic fever in the past few decades, the virus's natural reservoir remains unknown, and concern about the possibility of airborne transmission remains.[64]

Reductions in public health infrastructure and the complexities associated with outsourcing of clinical care and certain public health functions all compound the problem of outbreak detection and control. Military preventive medicine specialists affected by military medical downsizing will be challenged to meet the need for rapid-response, multidisciplinary teams to direct investigations of highly dynamic events. These challenges call for the rapid adoption of new technology for team communication, field laboratory analysis, specimen holding and processing, and further automation of data analysis. As the scope of potential problems increases, major new prevention modalities (eg, food irradiation) will be adopted and will need to be evaluated. Vital partnerships between the Department of Defense, the CDC, the National Institutes of Health, the Food and Drug Administration, the Department of Agriculture, the Environmental Protection Agency, and the World Health Organization will have to be productive, cooperative relationships with clearly delineated roles and responsibilities to achieve maximum efficiency and effectiveness. Telecommunication, telemedicine, and computer modeling need to be evaluated as potential additions to the suitcase of the shoeleather epidemiologist, who is now working on outbreaks that can spread over continents. Ultimately, the outcome of these futuristic investigations should be the same as those of today: new health policies for prevention based on supportive data proven valid by rigorous hypothesis testing.

REFERENCES

1. Rammelkamp CH, Wannamaker LW, Denny FW. The epidemiology and prevention of rheumatic fever. *Bull NY Acad Med*. 1952;28:321–334.

2. Joseph PR, Millar JD, Henderson DA. An outbreak of hepatitis traced to food contamination. *N Engl J Med*. 1965;273:188–194.

3. Wallace MR, Garst PD, Papadimos TJ, Oldfield EC. The return of acute rheumatic fever in young adults JAMA. 1989;262:2557–2561.

4. Duchin JS, Koster FT, Peters CJ, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. *N Engl J Med*. 1994;330:950–955.

5. Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the Washington experience. *JAMA*. 1994;272:1349–1353.

6. Centers for Disease Control and Prevention. Update: outbreaks of *Cyclospora cayetanensis* infections—United States and Canada, 1996. *MMWR*. 1996;45:611–612.

7. Feighner BH, Pak SI, Novakoski WL, Kelsey LL, Strickman D. Reemergence of *Plasmodium vivax* malaria in the Republic of Korea. *Emerg Infect Dis*. 1998;4:295–297.

8. Bjorkman A, Phillips-Howard PA. The epidemiology of drug-resistant malaria. *Trans R Soc Trop Med Hyg*. 1990;84:177–180.

9. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. The emergence of drug-resistant tuberculosis in New York City. *N Engl J Med*. 1993;328:521–526.

10. Foege WH. Alexander D. Langmuir—his impact on public health. *Am J Epidemiol*. 1996;144(8):S11–S15.

11. Okumura T, Takasu N, Ishimatsu S, et al. Report on 640 victims of the Tokyo subway sarin attack. *Ann Emerg Med*. 1996;28:129–135.

12. Henderson DA. Bioterrorism as a public health threat. *Emerg Infect Dis*. 1998;4:488–492.

13. Centers for Disease Control. Case definitions for infectious conditions under public health surveillance. *MMWR*. 1997;46(RR-10):1–58.

14. Wegman DH, Woods NF, Bailar JC. Invited commentary: how would we know a Gulf War syndrome if we saw one? *Am J Epidemiol*. 1997;146:704–711.

15. Hyams KC, Roswell RH. Resolving the Gulf War syndrome question. *Am J Epidemiol*. 1998;148:339–342.

16. Davis JP, Chesney PJ, Wand PJ, LaVenture M. Toxic-shock syndrome: epidemiologic features, recurrence, risk factors, and prevention. *N Engl J Med*. 1980;303:1429–1435.

17. Gray GC, Knoke JD, Berg SW, Wignall FS, Barrett-Connor E. Counterpoint: responding to suppositions and misunderstandings. *Am J Epidemiol*. 1998;148:328–333.

18. Olson PE, Bone WD, LaBarre RC, et al. Coccidioidomycosis in California: regional outbreak, global diagnostic challenge. *Mil Med*. 1995;160:304–308.

19. Last JM, ed. *A Dictionary of Epidemiology*. 3rd ed. New York: Oxford University Press, 1995.

20. Croft A, Smith H, Creamer I. A pseudo-outbreak of skin disease in British troops. *J R Soc Med*. 1996;89:552–556.

21. Dunn D. Health care 1999: a national bellwether. *J Health Care Finance*. 1996;22(3):23–27.

22. Burki DR, Bernasconi C, Bodmer T, Telenti A. Evaluation of the relatedness of strains of *Mycobacterium avium* using pulsed-field gel electrophoresis. *Eur J Clin Microbiol Infect Dis*. 1995;14:212–217.

23. Wurtz R, Demarais P, Trainor W, et al. Specimen contamination in mycobacteriology laboratory detected by pseudo-outbreak of multidrug-resistant tuberculosis: analysis by routine epidemiology and confirmation by molecular technique. *J Clin Microbiol*. 1996;34:1017–1019.

24. Whelen AC, Persing DH. The role of nucleic acid amplification and detection in the clinical microbiology laboratory. *Annu Rev Microbiol*. 1996;50:349–373.

25. Centers for Disease Control and Prevention. Outbreaks of pseudo-infection with Cyclospora and Cryptosporidium—Florida and New York City, 1995. *MMWR*. 1997;46:354–358.

26. Centers for Disease Control. Recommendations for collection of laboratory specimens associated with outbreaks of gastroenteritis. *MMWR*. 1990;39(RR-14):1–13.

27. Longfield JN, Winn RE, Gibson RL, Juchau SV, Hoffman PV. Varicella outbreaks in Army recruits from Puerto Rico: varicella susceptibility in a population from the tropics. *Arch Intern Med*. 1990;150:970–973.

28. Jones WHS, ed. *Hippocrates: Airs, Waters, Places*. Cambridge, Mass: Harvard University Press; 1948.

29. Centers for Disease Control. Hospital-associated outbreak of *Shigella dysenteriae* type 2—Maryland. *MMWR*. 1983;32:250–252.

30. Centers for Disease Control. Acute rheumatic fever among Army trainees—Fort Leonard Wood, Missouri, 1987–1988. *MMWR*. 1988;37:519–522.

31. Pan American Health Organization. *Guidelines for the Establishment of Systems for the Epidemiological Surveillance of Food-borne Diseases and the Investigation of Outbreaks of Food Poisoning*. PAHO, Division of Communicable Diseases Prevention and Control; 1993.

32. *Laboratory Sample Submission Guide*. Ft. Sam Houston, Tex: Dept of Defense Veterinary Laboratory; 1998.

33. DeFraites RF. *Preliminary Report of* Campylobacter enteritis *Outbreak*. Washington, DC: Walter Reed Army Institute of Research; 1990. Memorandum, 14 June 1990.

34. International Association of Milk, Food and Environmental Sanitarians. *Procedures to Investigate Waterborne Illness*. 2nd ed. Des Moines, Iowa: IAMFES; 1996.

35. Blacklow NR, Greenberg HB. Viral gastroenteritis. *N Engl J Med*. 1991;325:252–264.

36. Centers for Disease Control and Prevention. Surveillance for waterborne-disease outbreaks—United States, 1993–1994. *MMWR*. 1996;45(SS-1):1–33.

37. Wenzel RP. A new hantavirus infection in north America. *N Engl J Med*. 1994;330:1004–1005.

38. Kahn HA, Sempos CT. *Statistical Methods in Epidemiology*. New York: Oxford University Press; 1989.

39. MacDonald KL, Osterholm MT. The emergence of *Escherichia coli* O157:H7 infection in the United States: the changing epidemiology of foodborne disease. *JAMA*. 1993;269:2264–2266.

40. Tauxe RV. Emerging foodborne diseases: an evolving public health challenge. *Emerg Infect Dis*. 1997;3:425–434.

41. Hennessy TW, Hedberg CW, Stutsker L, et al. A national outbreak of *Salmonella enteriditis* infections from ice cream. *N Engl J Med*. 1996;334:1281–1286.

42. Blaser MJ. How safe is our food? Lessons from an outbreak of salmonellosis. *N Engl J Med*. 1996;334:1281–1286.

43. Centers for Disease Control and Prevention. Hepatitis A associated with consumption of frozen strawberries—Michigan, March 1997. *MMWR*. 1997;46:288,295.

44. Kolavic SA, Kimura A, Simons SL, Slutsker L, Barth S, Haley CE. An outbreak of *Shigella dysenteriae* type 2 among laboratory workers due to intentional food contamination. *JAMA*. 1997;278:396–398.

45. Torok TJ, Tauxe RV, Wise RP, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA*. 1997;278:389–395.

46. Wiener SL, Barratt J. Biological warfare defense. In: *Trauma Management for Civilian and Military Physicians*. Philadelphia: WB Saunders; 1986: 508–509.

47. McDade JE, Franz D. Bioterrorism as a public health threat. *Emerg Infect Dis*. 1998;4:493–494.

48. Meselson M, Guillemin V, Hugh-Jones M, et al. The Sverdlovsk anthrax outbreak of 1979. *Science*. 1994;266:1202–1208.

49. Nozaki H, Hori S, Shinozawa Y, et al. Secondary exposure of medical staff to sarin vapor in the emergency room. *Intensive Care Med*. 1995;21:1032–1035.

50. Aiken LR. *Questionnaires and Inventories: Surveying Opinions and Assessing Personality*. New York: John Wiley & Sons; 1997.

51. Aday L. *Designing and Conducting Health Surveys*. 2nd ed. San Francisco: Jossey-Bass Publishers; 1996.

52. Levine RJ. *Ethics and Regulation of Clinical Research*. 2nd ed. Baltimore: Urban & Schwarzenberg Publishers; 1986.

53. Presidential Decision Directive 39; 1996.

54. Osterholm MT. Cyclosporiasis and raspberries—lessons for the future. *N Engl J Med*. 1997;336:1597–1599.

55. Schlesselman JJ. *Case Control Studies: Design, Conduct, Analysis*. New York: Oxford University Press; 1982.

56. Gregg MB. *Field Epidemiology*. New York: Oxford University Press; 1996.

57. Barg NL. An introduction to molecular hospital epidemiology. *Infect Control Hosp Epidemiol*. 1993;14:395–396.

58. Goering RV. Molecular epidemiology of nosocomial infection: analysis of chromosomal restriction fragment patterns by pulsed-field gel electrophoresis. *Infect Control Hosp Epidemiol*. 1993;14:595–600.

59. Tompkins LS. The use of molecular methods in infectious diseases. *N Engl J Med*. 1992;327:1290–1297.

60. Kanesa-thasan N, Chang GJ, Smoak BL, Magill A, Burrous MJ, Hoke CH Jr. Molecular and epidemiologic analysis of dengue virus isolates from Somalia. *Emerg Infect Dis*. 1998;4:299–303.

61. Reingold AL. Outbreak investigations—a perspective. *Emerg Infect Dis*. 1998;4:21–27.

62. Herwaldt BL, Ackers ML, et al. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. *N Engl J Med*. 1997;336:1548–1556.

63. Centers for Disease Control and Prevention. Malaria among U.S. military personnel returning from Somalia, 1993. *MMWR*. 1993;42:524–526.

64. Breman JG, van der Groen G, Peters CJ, Heymann DL. International Colloquium on Ebola Virus Research: summary report. *J Infect Dis*. 1997;176:1058–1063.