Chapter 2

EPIDEMIOLOGY OF BIOWARFARE AND BIOTERRORISM

ZYGMUNT F. DEMBEK, PHD, MS, MPH, LHD*; JULIE A. PAVLIN, MD, PHD, MPH†; MARTINA SIWEK, PHD‡; AND MARK G. KORTEPETER, MD, MPH§

*Colonel (Retired), Medical Service Corps, US Army Reserve; Associate Professor, Department of Military and Emergency Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814; formerly, Chief, Biodefense Epidemiology and Education & Training Programs, Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland

†Colonel (Retired), Medical Corps, US Army; Deputy Director, Armed Forces Health Surveillance Center, 11800 Tech Road, Silver Spring, Maryland 20904

‡Chief Scientist, Cherokee Nation Technology Solutions in Support of Global Emerging Infections Surveillance and Response System, Armed Forces Health Surveillance Center, 11800 Tech Road, Silver Spring, Maryland 20904; formerly, Science Manager/Liaison, Biosurveillance Management Office, Joint Program Executive Office, Aberdeen Proving Ground, Maryland

§Colonel, Medical Corps, US Army; Director, Infectious Disease Clinical Research Program, Department of Preventive Medicine, Associate Dean for Research, Associate Professor of Preventive Medicine and Medicine, Consultant to the Army Surgeon General for Biodefense, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814; formerly, Deputy Commander, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland

A portion of this chapter has previously been published as: Dembek ZF, Kortepeter MG, Pavlin JA. Discernment between deliberate and natural infectious disease outbreaks. *Epidemiol Infect*. 2007;135:353–371.

INTRODUCTION

Preparing for and responding to biological warfare (BW) or bioterrorism (BT) is a public health issue and falls within the purview of public health professionals, because preparation for natural disease outbreaks has the dual benefit of BW/BT preparation. An understanding of basic epidemiology is needed before, during, and after an event to identify populations at risk, target preventive measures such as vaccinations, recognize an outbreak, track and limit disease spread, and provide postexposure treatment or prophylaxis. Many disease-specific management needs such as vaccination and prophylaxis are discussed elsewhere and are not considered here. Also, agricultural terrorism is discussed in chapter 3. This chapter will focus on detection and epidemiological investigation including distinguishing between natural and intentional events. Brief case studies will be presented to demonstrate important indicators and lessons learned from historical outbreaks. Finally, traditional methods of surveillance and ways to improve surveillance for BW/BT will be discussed.

THE EPIDEMIOLOGY OF EPIDEMICS

Definition

The word epidemic comes from the Greek "epi" and "demos," meaning "upon a mass of people assembled in a public place."[1] An epidemic is defined as the occurrence in a community or region of an unusually large or unexpected number of disease cases for the given place and time.[2] Therefore, a critical foundation is knowing baseline rates of disease to determine whether an epidemic is occurring. This information can be at the local, regional, national, or global level, and can be seasonal. As an example, thousands of influenza cases in January in the United States may not be unusual; however, thousands of cases in the summer may be cause for concern, similar to what was seen with an early summer wave of cases of H1N1 swine variant influenza in 2009. Also, even a single case of a rare disease can be considered an epidemic. With the absence of a woolen mill industry in the United States, any inhalational anthrax case should be highly suspect. Many of the diseases considered as classic BW agents, such as smallpox (considered to be eradicated), viral hemorrhagic fevers, and pneumonic plague are rare, and a single case should be investigated. Determining whether an outbreak occurs depends, therefore, on the disease, the at-risk population, the location, and the time of year.

For an outbreak to occur, three points of the classic epidemiological triangle must be present (Figure 2-1). There must be a pathogen or agent, typically a virus, bacterium, rickettsia, fungus, or toxin, and a host (in this case, a human) who is susceptible to that pathogen or agent. The two need to be brought together in the right environment to allow infection of the host directly by another individual, by a vector, or through another vehicle, such as food, water, or contact with fomites (inanimate objects). The environment must also permit potential transmission to other susceptible hosts. Disruption of any of these three points of the triangle can limit or disrupt the outbreak; therefore, it is important to know and understand the characteristics of the three for any specific disease to control an epidemic. For example, if potential hosts are vaccinated, disease spread would be significantly limited or if the environment is modified, spread may also be limited (eg, cleaning up garbage around a home limits rat food and harborage, and thus minimizes the risk of contact with fleas capable of transmitting plague).[3]

Recognition

Immediate effects on humans and possibly the environment are evident when an explosion occurs or a chemical weapon is released. However, because of the incubation periods of infectious pathogens, release of a BW/BT agent may be silent and the casualties produced after a release may be dispersed in time and space to primary care clinics and hospital emergency departments. Even toxins have latent periods prior to symptom onset. Therefore, the success in managing a biological event hinges directly on whether and when the event is recognized.

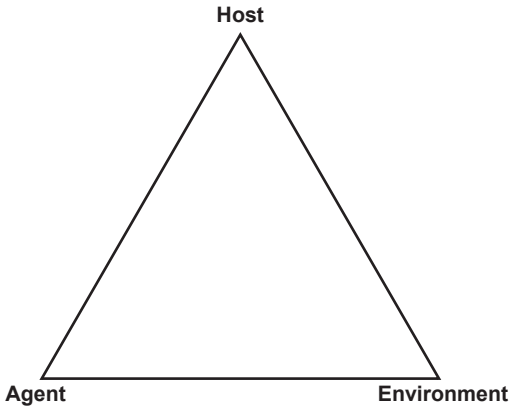

Figure 2-1. The epidemiological triangle

An example of the ramifications of delayed disease outbreak recognition occurred in 1972 in the former Yugoslavia. A single unidentified smallpox case led to 11 secondary cases, also unrecognized. Within a few weeks there was an outbreak of 175 smallpox cases and 35 deaths that led to a massive vaccination effort and border closure.[4] Early disease recognition may have significantly modified the outcome. Modeling studies of a BT-caused smallpox outbreak have shown that the more rapidly a postrelease intervention occurred, including quarantine and vaccination, the greater the chances that intervention would halt the spread of disease.[5–7] When medical professionals identify a new case, it is unlikely that a BW/BT event would be the first cause suspected, especially if the disease presents similar to other diseases that might occur simultaneously, such as influenza. Clinicians generally consider the source to be a common endemic disease at first. Alternative considerations might include a new or emerging disease, or a laboratory accident before considering BW/BT.[8] Therefore, care providers should be familiar with the diseases of BW/BT that could be spread intentionally and maintain a healthy "index of suspicion" to recognize an event early enough to significantly modify the outcome.[9] Furthermore, although the government has generated lists of potential threat agents, public health authorities must be mindful that a perpetrator does not necessarily follow any list and may choose an organism based on access or some other unanticipated reason. Also, a perpetrator might listen to government and other media information, and respond accordingly, thereby undermining a government terrorism response.

Clinicians, hospital infection control personnel, school or healthcare facility nursing staff, laboratory personnel, and other public health workers have a responsibility to notify public health authorities about disease outbreaks. State and local public health officials regularly examine and review disease surveillance information to detect outbreaks in a timely manner and provide information to policymakers on disease prevention programs. Time constraints are inherent in obtaining case report information because of the elapsed time from patient presentation, lab specimen collection and submission, and laboratory testing time, to final disease or organism reporting. Furthermore, the initial BW/BT disease recognition may not come from a traditional reporting partner or surveillance method. Instead, pharmacists and clinical laboratory staff who receive requests or samples from numerous healthcare providers may be the first to note an increase in purchases or prescriptions of certain medications (eg, antibiotics or antinausea or diarrheal agents) or orders for certain laboratory tests (eg, sputum or stool cultures), respectively. Also, because many of the category A high-threat diseases are zoonoses (primarily infect animals), with humans serving as accidental hosts, veterinarians may be the first to recognize the disease in animals prior to the ensuing human disease. Media and law enforcement personnel and other nontraditional reporters of outbreaks may also provide information on a BT event or potential cases. Therefore, it is important for all those different types of individuals to maintain the same index of suspicion as healthcare providers for unusual events in their respective fields.

Potential Epidemiological Clues to an Unnatural Event

It is often not possible to determine the objectives of a BT perpetrator in advance, whether the intent is to kill, incapacitate, or obtain visibility. It also may be difficult to discern how a biological agent was dispersed, whether through the air, in contaminated food or water, or by direct inoculation. In a biological attack, the number of casualties may be small and therefore unrecognized as intentionally infected, especially if the agent is a common cause of disease in the community. In addition, given the agent's incubation period, individuals may seek care from different care providers or travel to different parts of the country before they become ill and seek medical care. Despite the potential for these situations to occur, it is useful for healthcare providers to be aware of potential clues that may be tip-offs or "red flags" of something unusual. Although these clues may occur with natural outbreaks and do not necessarily signal a BW/BT attack, they should at least heighten suspicion that something out of the ordinary is occurring. The following compilation is an illustrative list; however, additional clues may be found elsewhere.[10,11]

Clue 1: A highly unusual event with large numbers of casualties. Although the mention of BW or BT may elicit images of massive casualties, they may not actually occur with a real BW/BT event. Numerous examples of naturally spread illness have caused massive casualties and some BW/BT events have few or no casualties. Nevertheless, the type of large outbreak that should receive particular attention is one in which no plausible natural explanation for the cause of the infection exists.

Clue 2: Higher morbidity or mortality than is expected. If clinicians are seeing illnesses that are causing a higher morbidity or mortality than what is typically seen or reported for a specific disease, this may indicate an unusual event. A perpetrator may have modified an agent to make it more virulent or selected antibiotic resistance in an organism usually

sensitive to antibiotics. Individuals could also be exposed to a higher inoculum than they would normally receive with natural spread of the agent, thus causing higher morbidity or mortality.

Clue 3: Uncommon disease. Many infectious diseases have predictable population and infectivity distributions based on environment, host, and vector factors; yet unnatural spread may occur if a disease outbreak is uncommon for a certain geographical area. Concern should be heightened if the naturally occurring disease requires a vector for spread and the competent vector is missing. For example, if a case of yellow fever, which is endemic to parts of South America and sub-Saharan Africa, occurred in the United States without any known travel, it would be a concern. Natural outbreaks have occurred in new geographical locations including the West Nile virus (WNV) in New York City in 1999.[12] It is important to consider whether the occurrence of these uncommon diseases is natural.

Clue 4: Point source outbreak. For any outbreak, it is useful to develop an epidemic curve demonstrating the timeline of dates when patients developed illness. These curves can have different morphologies depending on whether individuals are exposed at the same time from a single source or over time, and whether the illness spreads from person to person. In an intentional BT event, a point source outbreak curve would most likely be seen[13] when individuals are exposed at a similar point in time. The typical point source outbreak curve has a relatively quick rise in cases, a brief plateau, and then an acute drop, as seen in Figure 2-2. For example, the epidemic curve might be slightly compressed after an aerosol release because infected individuals were exposed more closely in time (ie, within seconds to minutes of each other) compared with individuals becoming ill after eating a common food over a period of hours. Or the inoculum may be greater than what is typically seen with natural spread, thus yielding a shorter incubation than expected. It should also be considered that the spread of a biological agent capable of being transmitted from person to person could result in a propagated (secondary transmission) outbreak, with a case distribution more similar to that depicted in Figure 2-3.

Clue 5: Multiple epidemics. If a perpetrator can obtain and release a single agent, it is also feasible that multiple perpetrators could release single or multiple agents at different locations. If simultaneous epidemics occur at the same or different locations with the same or multiple organisms, an unnatural source must be considered. It must also be considered that a mixture of biological organisms with different disease incubation periods could be released, and thus would cause simultaneous or serial outbreaks of different diseases in the same population.

Clue 6: Lower attack rates in protected individuals. This clue is especially important for military personnel. If certain military units had some type of respiratory protection, such as mission-oriented protective posture gear or high-efficiency particulate air-filtered masks, or stayed in a high-efficiency particulate air-filtered tent and had lower rates of illness than nearby groups that were unprotected, this may indicate that a biological agent has been released via aerosol.

Clue 7: Dead animals. Historically, animals have been used as sentinels of human disease. The storied use of canaries in a coal mine to detect the presence of noxious gases is one example. This phenomenon was observed during the naturally occurring WNV outbreak in New York City in 1999, when many of the local crows, along with the exotic birds at the Bronx Zoo, developed fatal disease.[14,15] Because many biological agents that could be used for BW/BT are zoonoses, a local animal die-off may also indicate a biological agent release that may also infect humans.

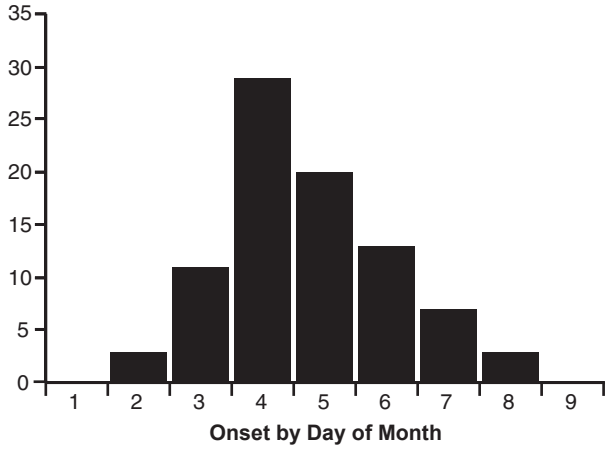

Figure 2-2. Typical point source outbreak epidemic curve

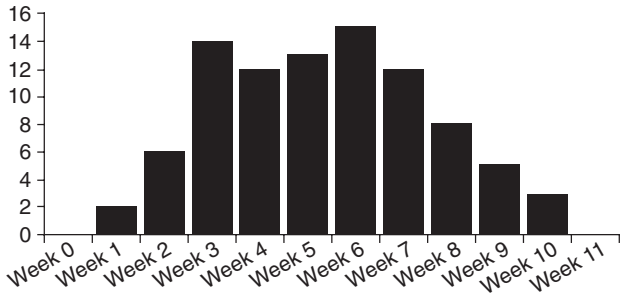

Figure 2-3. Typical continuous common source outbreak epidemic curve

Clue 8: Reverse or simultaneous spread. Zoonotic illnesses exhibit a typical pattern: an epizootic first occurs among a susceptible animal population, followed by cases of human illness. With anthrax, one would expect ill animals to be identified before cutaneous disease in workers processing the animals or before gastrointestinal disease in people who may have eaten meat from the infected animals. After the accidental release of anthrax spores in Sverdlovsk (see description and case review of the 1979 Sverdlovsk anthrax outbreak), an outbreak occurred simultaneously in people and animals downwind of the weapons facility.[16] If human disease precedes animal disease or human and animal disease are simultaneous, then unnatural spread should be considered.

Clue 9: Unusual disease manifestation. More than 95% of worldwide anthrax cases are cutaneous illness. Therefore, a single case of inhalational anthrax should be considered highly suspicious for BW/BT until proven otherwise. The rare exception is an inhalational anthrax case in a woolen mill worker or in someone handling animal skins from endemic areas, which has recently occurred.[17] This logic may be applied to cases of a disease such as plague, where the majority of naturally occurring cases are the bubonic, not the pneumonic form.

Clue 10: Downwind plume pattern. The geographic locations where cases occur can be charted on a geographic grid or map. If the reported cases appear clustered in a downwind pattern, then an aerosol release may have occurred. During the investigation into the anthrax outbreak in Sverdlovsk in 1979 (as examined later in this chapter), mapping out case locations helped to determine that the anthrax cases were caused by an aerosol release rather than a contaminated food source.[16]

Clue 11: Direct evidence. The final clue may be the most obvious and the most useful. Determining the intentional cause of illnesses is easier if a perpetrator leaves a "signature" or direct evidence of a biological attack. Such a signature could be a letter filled with anthrax spores,[18] a spray device or another vehicle for agent spread, or claims by a person or group of a biological attack. It would be useful to compare samples from any found device with the clinical samples obtained from victims to verify that they are the same organism.

EXHIBIT 2-1

GOALSOFANOUTBREAKINVESTIGATION

- Find the source of disease.
- Rapidly identify cases.
- Prevent additional cases through implementation of appropriate control measures.
- Identify strategies to prevent further outbreaks.
- Evaluate existing prevention strategies (including control measures immediately put into place).
- Address public concerns.
- Provide information to leadership to support informed decisions.
- Improve scientific knowledge about the disease.

Outbreak Investigation

It is important to understand the basic goals of an outbreak investigation, as seen in Exhibit 2-1. Any outbreak (a greater than expected number of cases in a specific location, group of people, or time period) should be investigated quickly to find the source of the disease. If an outbreak is ongoing, the source of infection needs to be identified and eliminated quickly. Even if the exposure source has dissipated, all cases should be identified expeditiously, so that ameliorative care can be offered and case interviews can be conducted. Case identification can assist in preventing additional cases, especially with a transmissible infectious disease. Providing information to the public and to leaders is also key to ensure the best public health policies are enacted and followed. With notification of any outbreak, whether natural or intentionally caused, there are standard steps to follow in an outbreak investigation (Exhibit 2-2), although these steps may not always occur in order.[19] The first step is preparation, which involves having the necessary response elements (personnel, equipment, laboratory capabilities) ready and establishing communications in advance with partners who may assist in the investigation. Once an event is ongoing, the second step is to investigate, verify the diagnosis, and decide whether an outbreak exists. Early in an outbreak, its significance and scope are often not known. Therefore, existing surveillance information and heightened targeted surveillance efforts are used to determine whether reported items are cause for concern.

The third step is to define the outbreak and seek a definitive diagnosis based on historical, clinical, epidemiological, and laboratory information. A differential diagnosis can then be established.

The fourth step is to establish a case definition that includes the clinical and laboratory features that the ill individuals have in common. It is preferable to use a

EXHIBIT 2-2

TEN STEPS IN AN OUTBREAK INVESTIGATION

1. Prepare for fieldwork (identify resources).
2. Verify the diagnosis. Determine whether an outbreak exists.
3. Define the outbreak and seek a diagnosis (including specimen collection and testing).
4. Develop a case definition and identify and count cases.
5. Develop exposure data with respect of person, place, and time.
6. Implement control measures and continually evaluate them.
7. Develop the hypothesis.
8. Test and evaluate the hypothesis with analytical studies and refine the hypothesis.
9. Formulate conclusions.
10. Communicate findings.

broad case definition at first and avoid excluding any potential cases too early. Objective clinical features are preferred, such as temperature exceeding 100.4°F, or diarrhea defined as greater than three watery bowel movements per day, as well as laboratory and pathological reports. The case definition enables the investigator to count cases and compare exposures between cases and noncases and compare these with other investigators and regions using the same case definition. To obtain symptom information, it may not be sufficient to look at healthcare facilities only, but also necessary to interview the ill persons and their family members, as well as coworkers, classmates, or others with whom they have social contact. It is important to maintain a roster of potential cases while obtaining this information. Commonly during an investigation, there is a risk of double or even triple counting cases because they may be reported more than once through different means. Key information needed from each ill person, besides identifying information to ensure accurate case counting and ability to contact the cases again if necessary, includes date of illness onset; signs and symptoms; recent travel; ill contacts at work, home, or school; animal exposures; and treatments received. With this information, an epidemic curve can be constructed (see Figure 2-2) that may provide information as to when a release may have occurred, especially if the disease is known, and an expected exposure date based on the typical incubation period, known ill contacts, or geographic risk factors.

Different modes of disease spread may have typical features that comprise an epidemic curve. If there is a common vehicle for disease transmission (such as a food or water source) that remains contaminated, it might be possible to see a longer illness plateau (a continuous common source curve [Figure 2-3]) than is seen with a point source of infection. If the agent is spread person to person, successive waves of illness may be seen as one group of individuals infects a follow-on group, which in turn infects another, and so on (Figure 2-4). With time and additional cases, the successive waves of illness may overlap with each other.

The fifth step is to document potential exposure data. Cases need to be identified and counted. Once cases have been identified, exposures based on person, place, and time can be determined. Obtaining information from individuals who would likely have had similar exposures but are not ill can also help determine the potential cause and method of an agent's spread. Information can be obtained either informally or formally with a case control study. A case control study is a type of study where investigators identify individuals with and without disease and compare their potential exposures or risk factors for disease. With a known exposure, one can also identify exposed and nonexposed populations and determine illness rates with a retrospective cohort study to help determine whether that particular exposure is a risk factor for disease.

The sixth step is to implement control measures as soon as feasible and continuously evaluate them. If necessary, control measures can be quickly implemented and then modified as additional case information becomes available. The seventh step is to develop a hypothesis. Based on the characteristics of the disease, the ill persons, and environmental factors, a hypothesis can usually be generated for how the disease occurred, how it is spreading, and the potential risk to the uninfected. The eighth step is to test and evaluate the hypothesis using analytical studies and refine the hypothesis.

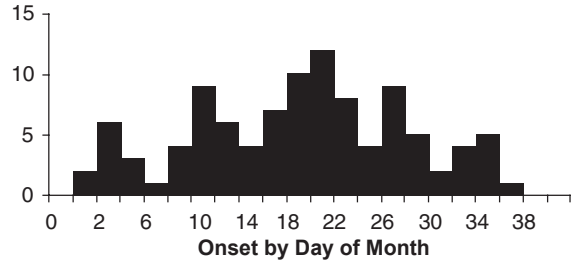

Figure 2-4. Typical propagated (secondary transmission) outbreak epidemic curve

Once developed, it is important to test the hypothesis to ensure it fits with the known facts. Does it explain how all the cases were exposed? It is possible that some outliers may seem as if they should be ill but are not, or some who are ill but have no known exposure. These outliers can sometimes be the key to determining what happened. With preliminary control measures implemented, the hypothesis can be tested formally with analytical studies. Further modifications in control measures might be needed and implemented.

The ninth step is to formulate a conclusion about the nature of the disease and exposure route. Findings can then be communicated (the tenth and final step) through the media or medical literature, depending on the urgency of notification to the public and medical community.

Experience from the anthrax mailings of 2001 indicates that during any BT event, intense pressure will be exerted on public health authorities to provide more information than is available.[20] As stated earlier, these distinct steps may not occur in sequence. It may be necessary to implement control measures with incomplete information, especially if an outbreak is fast moving or has a high morbidity or mortality rate. Whether the control measures appear to limit the disease spread or the casualty toll is the ultimate test of the accuracy of the original hypothesis.

Early in an investigation, it will probably not be known or suspected that an outbreak was unnaturally spread. Therefore, with a few exceptions, the investigation of an unnaturally spread outbreak will not differ significantly from the investigation of a naturally occurring outbreak. Public health authorities will work on both types of outbreaks. The significant difference is that, with a purposeful outbreak, a potential criminal event may have occurred. An additional goal of this type of investigation, under the purview of law enforcement personnel, is to bring the perpetrator to justice. Therefore, law enforcement personnel need to partner with public health officials as early as possible in any suspected BT case.[21]

Public health authorities must become familiar with the use of chain of custody, the process used to maintain and document the chronological history of the evidence, so that medical evidence/clinical samples or environmental samples obtained in the investigation will be admissible in a court of law. Environmental and biological samples can be crucial in determining whether a deliberate release of a pathogen has occurred (see the case study in this chapter about the release of *Bacillus anthracis* in Tokyo by the Aum Shinrikyo).

Although chain of custody is important, public safety should be the primary concern. Public health authorities must also have an open mind for unusual modes of disease spread, being especially careful to ensure their personnel's safety if a potential exposure risk occurs during the investigation. Public health authorities conducting a field investigation should have personal protective equipment and be trained in its proper use, and they should also have access to occupational health resources if pre- or postexposure prophylaxis or monitoring is needed.

EPIDEMIOLOGICAL CASE STUDIES

The following epidemiological case studies are presented to demonstrate the differences between naturally occurring and purposefully created epidemics. Biological attacks and some naturally occurring epidemics of historical significance are considered in the context of BT. Some purposeful BT events have not caused illness; however, some naturally occurring outbreaks were initially considered as potential BT events because of the particular disease or nature of clinical case presentation.

Public health authorities could be held accountable to make a determination quickly as to whether an infectious disease outbreak has been purposefully caused, yet they may lack the necessary information because there may not be clear evidence or responsibility claimed for a BT event. A thorough understanding of how to investigate suspect outbreak occurrences may better enable public health authorities to make difficult public health policy decisions.

Bioterrorism Events

The following section describes BT incidents that occurred in the United States and Japan. None of these events was immediately recognized as having been intentional. The 2001 mail-associated anthrax outbreak and mail-associated ricin attack were recognized within days to weeks. With new sensors installed in mail collection facilities, mailings of ricin in 2013 were recognized immediately. However, for previous BT incidents (anthrax and glanders in 1915, salmonellosis in 1984, and anthrax in 1995), intentionality was not recognized for a year or longer after the initial event.

Anthrax and Glanders—Maryland; New York, New York; and Virginia, 1915–1916

From 1915 through 1918, Germany had a state-sponsored offensive BW program to sabotage suppliers to the Allies directed at draft, cavalry, and military

livestock. Human disease was neither intended nor recorded from these events, although the program could have been expanded to spread zoonotic illness among a target population. Unintended human disease may have occurred but was never recorded. Countries targeted by Germany included the United States, Argentina, Romania, Russia, Norway, and Spain. The German army general staff directed and implemented the biological sabotage program despite official German army doctrine prohibiting such activities. Germany's plans to spread a wheat fungus and contaminate food produced at "meat factories" were dropped.[22] One 1916 German plan never carried out proposed to drop vats of plague cultures from Zeppelins over England.[23]

In April 1915 German-American physician Anton Dilger returned to the United States from Germany with cultures of *Burkholderia mallei* and *Bacillus anthracis*. His intent was to infect horses and mules being shipped from the United States to France and England for use in cavalry and transport. These cultures were propagated and tested for virulence using guinea pigs in the basement of a house (known as "Tony's Lab") rented by Anton and his brother, Carl, in Chevy Chase, Maryland, near Washington, DC.[24] From the summer of 1915 through the fall of 1916, the cultures were used to infect horses and mules in holding pens in docks at the ports of Baltimore, Maryland; Newport News, Virginia; Norfolk, Virginia; and New York, New York. Stevedores working for German steamships were recruited and given 2-inch, cork-stoppered glass vials containing the cultures, in which a hollow steel needle had been placed. These stevedores were instructed to wear rubber gloves while jabbing the animals with the needle. These cultures were also spread to the animals by pouring them into the animal feed and drinking water.[25,26]

Case Review of 1915–1916 Anthrax and Glanders Incidents

Biological Agents: *B anthracis*, gram-positive bacillus; *B mallei*, gram-negative bacillus

Potential Epidemiological Clues: 2, 7, 8

Review: A full assessment of the success of this BW program 90 years later is not possible. German agents claimed that epidemics occurred among the animals shipped from the US ports. However, disease observed among animals might have originated naturally or from stressful holding and shipment conditions.

Few surveillance systems incorporate comprehensive veterinary surveillance. This is an important disease detection vulnerability because many BW agents (ie, *B anthracis*, *Brucella suis*, *B mallei*, *Burkholderia pseudomallei*, *Coxiella burnetii*, *Francisella tularensis*, *Yersinia pestis*, encephalitis, and hemorrhagic fever viruses) can cause zoonotic illness.

Lessons Learned: Veterinarians discovering glanders or anthrax and other US Department of Agriculture (USDA) select agricultural agents in livestock should report these diseases to state health and federal authorities as possible BT indicators.[27,28]

A comprehensive animal surveillance network would include reports from veterinary examinations of farm and companion animals, and from wildlife examinations by state environmental officials and animal rehabilitators. Current animal disease surveillance networks that address these deficiencies include the National Animal Health Laboratory Network[29] and the Centers for Epidemiology and Animal Health,[30] both part of the USDA.

Salmonellosis—The Dalles, Oregon, 1984

A large outbreak of Salmonella cases occurred in and around The Dalles, Oregon, in 1984. This farming community, with a 1984 population of 10,500, is near the Columbia River on the border of Oregon and Washington. Salmonellosis is the second most common bacterial foodborne illness and is underreported by a factor of about 38-fold.[31,32] The average onset period for salmonellosis is about 12 to 36 hours, and the disease manifests as acute gastroenteritis. Fever occurs, anorexia and diarrhea persist for several days, and more severe manifestations may at times occur, especially in very young or elderly persons. Contaminated food (most often poultry) is the principal route of disease transmission.[33]

Given its high incidence in the United States, public health authorities would not normally consider a foodborne salmonellosis outbreak as intentional. It has been estimated that 1.4 million salmonellosis infections occur annually in the United States, resulting in 15,000 hospitalizations and 400 deaths.[34] Therefore, the index of suspicion for an intentional Salmonella outbreak was—and remains—low. However, atypical events associated with this outbreak eventually led officials to realize that this particular disease occurrence was historically different. Two cohorts of cases occurred: (1) from September 9 through 18, 1984, and (2) from September 19 through October 10, 1984. Public health authorities received initial reports of illness on September 17, and local and state health officials interviewed the ill persons. Patronizing two restaurants in the city of The Dalles and eating salad bar food items were commonly cited in these interviews. *Salmonella typhimurium* isolates were then obtained from clinical specimens from the ill persons.[35]

The source for this outbreak was puzzling. Epidemiological analysis revealed multiple items rather than a single suspect item as the cause of the restaurant patrons' illness. This finding is not uncommon either during the initial stages of an investigation of a foodborne disease outbreak (until a suspected food item is identified), or when an infected food handler is identified as the source of the outbreak. Although dozens of food handlers became ill, their time of

symptom onset did not precede those of their customers. As gastroenteritis cases occurred in increasing numbers, health officials imposed a closure of all salad bars in The Dalles on September 25. By the end of the outbreak, 751 salmonellosis cases were identified, with those affected ranging in age from newborns to 87 years, and most were associated with dining in 10 area restaurants. At least 45 persons were hospitalized, but no fatalities occurred.

Bhagwan Shree Rajneesh, a charismatic guru, had established a community for his followers in 1981 at a ranch near The Dalles. These cult members, or "Rajneeshees," attempted to use Oregon's liberal voter registration laws to control zoning and land use restrictions to their advantage. Conflict between the commune and the neighboring traditional community had escalated. To gain political control of the area, the Rajneeshees attempted to influence an election by making voters too ill to vote.[22] Approximately 12 individuals were involved in the plot, and up to 8 individuals distributed *S typhimurium* cultures to the salad bars. After considering the use of several biological agents, including *Salmonella typhi* (the causative agent of typhoid fever) and the human immunodeficiency virus, the Rajneeshees legally obtained cultures of *S typhimurium* (American Type Culture Collection strain 14028) from a commercial supplier and used them to grow bacterial stock cultures. The Rajneeshees first spread Salmonella by contaminating the commune members' hands to greet outsiders, as well as the county courthouse's doorknobs and urinal handles; these efforts did not cause illness. The cult also spread Salmonella cultures on salad bars in area restaurants.

Public health authorities conducted an extensive investigation in response to the salmonellosis outbreak. Authorities identified confirmed cases microbiologically by stool culture of *S typhimurium*, or with the clinical criteria of diarrheal illness and at least three of the following symptoms: fever, chills, headache, nausea, vomiting, abdominal pain, or bloody stools. *S typhimurium* was isolated from 388 patients. In the 4 years before the outbreak, the local health department had collected 16 isolates of Salmonella, 8 of which were *S typhimurium*. No local cases of salmonellosis had been reported in 1984 before August.[35]

The 38 restaurants in The Dalles were grouped according to the number of culture-confirmed customer cases with a single restaurant exposure in the week before symptom onset. Additional ill customers were located through laboratory reporting of clinical specimens or clinician reporting to public health authorities (passive disease surveillance). Press releases were issued to encourage disease reporting by patients and clinicians.[35] Public health officials interviewed ill persons to obtain their symptoms, risk factors, and comprehensive food histories, as well as the names of all persons who had eaten with them at the restaurant. Restaurant employees with the greatest number of cases were interviewed twice and required to submit a stool sample as a condition of continued employment. The state public health laboratory serotyped the Salmonella isolates and performed antibiotic-susceptibility testing on a subset. A representative sample of outbreak isolates was sent to the Centers for Disease Control and Prevention (CDC) for further characterization, during which the outbreak strain was compared with national surveys of human and veterinary isolates. Sanitarians inspected the restaurants, and tap water was collected and analyzed. The local health department and USDA also investigated the food distributors and suppliers used in these restaurants. None was found to have contaminated food, nor was a common supplier found for all of the implicated restaurants.

Many food items served at the salad bars of the restaurants were associated with illness and differed among the restaurants. Illness was associated with eating blue cheese dressing at one of the restaurants. The consumption of potato salad had the greatest association with illness, followed by lettuce. *S typhimurium* was isolated from the blue cheese dressing collected at one restaurant, but not from the dry mix used to prepare the dressing.

The size and nature of the outbreak eventually helped to initiate a criminal investigation. The source and cause of the outbreak only became known when the Federal Bureau of Investigation (FBI) investigated the cult for other criminal violations.[36] An Oregon public health laboratory official accompanying the FBI discovered an open vial containing the original culture strain of *S typhimurium* in the Rajneeshee clinic laboratory in October 1985.[22,35] This strain was indistinguishable from the outbreak strain as isolated from food items and clinical specimens, and records were found documenting its purchase before the outbreak.[35]

Intentional contamination of the salad bars is consistent with the retrospective epidemiology.[35] Eventually two cult members were arrested and served federal prison terms. Despite the Rajneeshees' success of the restaurant-associated BT, the publicity and subsequent legal pressure caused them to abandon subsequent efforts.[22]

Case Review of 1984 Salmonellosis Outbreak

Biological Agents: *S typhimurium*, gram-negative bacillus

Potential Epidemiological Clues: 1, 4, 5, 11

Review: Public health authorities found no statistical association with any single food item.[22] The isolation of *S typhimurium* from the blue cheese dressing, but not from the dry mix used in dressing preparation, should have indicated to authorities the contamination of the prepared dressing that was then served at a salad bar.

The ongoing law enforcement investigation eventually revealed purposeful restaurant food contamination by the Rajneeshees more than a year after the outbreak occurred.

Public health and law enforcement authorities lacked cooperative protocols in 1984; however, law enforcement teams in Oregon worked together with public health.

An outbreak of this magnitude now would initiate a joint inquiry and investigation by public health and law enforcement, increasing chances that the outbreak cause would be identified in a timelier manner.

Lessons Learned: These events illustrate the need to have joint public health and law enforcement investigations and mutual cooperation.

This outbreak shows t yohe importance of the mode of disease spread in discerning the source.

Although not occurring in this case, when different geographic locations are affected, there could be a central supplier of a contaminated product shipped to all the locations. Since there was not a single supplier in this situation, this served as a red flag that multiple contaminations may have occurred.

Anthrax—Tokyo, Japan, 1995

Sarin is a chemical (nerve) agent that causes blocking of the postsynaptic enzyme that degrades acetylcholine, thus leading to excessive salivation, lacrimation, respiratory compromise, and seizures. Many may be familiar with it as a result of its use in the Syrian civil war in 2014. The notorious sarin attacks in a Tokyo suburb, Kameido, in 1994 and 1995, culminated with a sarin release in the Tokyo subway system.[37,38] Less well known is that before its efforts with chemical weapons, the apocalyptic cult Aum Shinrikyo appears to have first invested efforts into producing biological agents and had attempted to use them.[22]

Shoko Asahara, a charismatic guru, built the Aum Shinrikyo cult into a membership of approximately 10,000 individuals with financial assets exceeding $300 million. Aum Shinrikyo's organization mimicked a government entity, with various ministries and departments, including a ministry of science and technology that included graduate-level researchers within modern laboratories interested in developing biological and chemical weapons. *B anthracis* cultures were also obtained and grown into a slurry for use as a biological weapon. This cult may have also investigated the use of *C burnetii* (the rickettsial organism that causes Q fever) and toxic mushrooms. In 1992 a team of 40 cult members, including Asahara, traveled to Zaire to attempt to acquire Ebola virus; the success of these efforts is unknown.

The Aum Shinrikyo experimented with the release of aerosolized biological agents. In June 1993 the cult sprayed *B anthracis* from the roof of one of its buildings in downtown Tokyo. In July 1993 the cult sprayed *B anthracis* from a moving truck onto the Diet (Japan's parliament) and also around the Imperial Palace in Tokyo.

Information about the anthrax releases became public when, during the arraignment of Asahara on May 23, 1996, for the Kameido sarin attack, cult members testified about their efforts to aerosolize a liquid suspension of *B anthracis* to cause an inhalational anthrax epidemic. Their goal was to have an epidemic trigger a world war that would permit Asahara to rule the world.[39] In 1999 a retrospective case-detection survey was conducted to assess the possibility that some anthrax cases may have been unreported. Complaints of odors from neighborhood residents were associated with the anthrax releases. These complaints were retrospectively mapped to provide the geographic areas of the greatest anthrax exposure risk. Physicians at 39 medical facilities serving this area were surveyed. None reported having seen cases of anthrax or relevant syndromes.[39] It is not known whether a similar retrospective examination of anthrax-caused animal deaths was or could have been performed. Danzig and colleagues wrote a comprehensive report that analyzed the Aum Shinrikyo's failures and successes in developing biological and chemical weapons.[40]

Case Review of 1995 Anthrax Releases
Biological Agents: *B anthracis*, gram-positive bacillus
Potential Epidemiological Clues: 11

Review: Technical errors in either the biological agent production or dissemination rendered the attacks harmless. In contrast, there were 12 deaths and about 1,000 hospitalizations from the sarin releases by the Aum Shinrikyo.[37]

Molecular analysis revealed that the *B anthracis* isolates were similar to the Sterne 34F2 strain, the strain of anthrax used in animal vaccines. Dispersal of this type of anthrax (regarded as nonpathogenic for immunocompetent individuals) had little possibility to cause harm to humans.[39]

Even if the strain was pathogenic, the concentration of spores in the liquid suspension is significantly less (104 bacteria/mL) than that considered optimal for a biological weapon (109–1010 bacteria/mL). The viscosity of the suspension was also problematic for successful aerosolization.[39]

The weather on the day of dispersal may have helped prevent infection: spore inactivation resulting from solar radiation could have further reduced the anthrax mix's potency.[39]

Lessons Learned: These experiences show that it is difficult to both create a pathogenic biological weapon and deploy it successfully.

Both health and law enforcement officials should be aware of the possibility for use of more than one biological agent or a combination of agents.

Environmental sample collection and proper storage are important for viability of pathogen cultures.

The then-emerging discipline of forensic molecular biology proved the occurrence of an anthrax release by analysis of archived samples 8 years after the incident.[41] The

contributions of advanced molecular techniques to the detection of BW and BT is examined in the section, Potential Impact of Advanced Molecular Techniques on the Epidemiology of Biowarfare and Bioterrorism, at the end of this chapter.

Shigellosis—Dallas, Texas, 1996

From October 29 through November 1, 1996, 12 clinical laboratory workers at the St Paul Medical Center in Dallas developed severe acute diarrheal illness.[22] *Shigella dysenteriae* type 2 was cultured from the stool of eight of these cases. This strain of shigella is uncommon and, before this outbreak, had last been reported as the source of an outbreak in the United States in 1983. A 13th individual became ill after eating pastries brought home by one of the laboratory workers; this individual also had stool cultures positive for *S dysenteriae* type 2. Five patients were treated in and released from hospital emergency departments and four were hospitalized, but no deaths resulted.[42]

During the subsequent epidemiological investigation,[43] laboratory employees who had worked during the first or third shifts, when the ill employees had worked, were interviewed. The employees stated that an unsigned email sent from a supervisor's computer invited recipients to take pastries available in the laboratory break room. The supervisor was away from the office when the email was sent, and the break room could only be accessed using a numeric security code. The muffins and pastries had been commercially prepared, yet no other cases in the community occurred outside of the hospital laboratory. The ill persons reported eating a pastry between 7:15 AM and 1:30 PM on October 29. Diarrhea onset for the ill laboratory workers occurred between 9:00 PM that day and 4:00 AM on November 1. The mean incubation period until diarrhea onset was 25 hours and was preceded by nausea, abdominal discomfort, and bloating. All who ate a muffin or doughnut became ill (ie, 100% attack rate). No increased risk for illness was found from eating food from the break room refrigerator or drinking any beverage, eating in the hospital cafeteria, or attending social gatherings during the estimated time of exposure to the pathogen.

An examination of the hospital laboratory storage freezer revealed tampering of reference cultures of *S dysenteriae* type 2. The stored reference cultures had each contained 25 porous beads that were impregnated with microorganisms. The *S dysenteriae* type 2 vial contained at that time only 19 beads, and laboratory records indicated that the vial had not been used. *S dysenteriae* type 2 was isolated in virtually pure culture from the muffin specimen, and the same organism was isolated from the stools of eight laboratory worker patients. Pulsed-field gel electrophoresis revealed that the reference culture isolates were indistinguishable from those obtained from a contaminated muffin and the collected stool cultures, but differed from two nonoutbreak *S dysenteriae* type 2 isolates obtained from other Texas counties during that time.

Case Review of 1996 Shigellosis Food Poisonings

Biological Agents: *S dysenteriae* type 2, gram-negative bacillus

Potential Epidemiological Clues: 3, 4, 11

Review: There was a strong epidemiological link among the ill persons, the cultured muffin, and the laboratory's stock culture of *S dysenteriae* type 2.

The pathogen provided important clues because it was known to be uncommon and no research with this microorganism had been conducted at the hospital; therefore, laboratory technicians were not at risk of infection through laboratory error. In addition, no concurrent outbreaks of *S dysenteriae* type 2 were reported nationally at the time.

Pastry contamination during commercial production was unlikely. Shigella contamination by a food service worker during food preparation would have had to occur subsequent to baking because Shigella bacteria would not have survived the heat.

When the epidemiological report was published,[42] it was hypothesized that someone had removed the laboratory culture of *S dysenteriae* type 2 from the freezer, cultured the microorganism and inoculated the pastries, and had access to the supervisor's computer and the locked break room.

On August 28, 1997, a laboratory technician who had access to the laboratory culture stocks and a history of purposeful use of biological agents against a boyfriend, was indicted on three charges of tampering with a food product, and accused of infecting 12 coworkers with *S dysenteriae* type 2. She was subsequently sentenced to 20 years in prison.

Lessons Learned: A match of clinical, food, and laboratory isolates helped to prove an epidemiological link among them. The knowledge that only postproduction tampering of the baked goods could have resulted in their successful contamination assisted with the investigation.

Anthrax—USA, 2001

On October 4, 2001, an inhalational anthrax case was reported in a 63-year-old man in Florida.[44] Public health and government authorities initially misunderstood the nature of inhalational anthrax exposure and assumed that he had contracted the illness by outdoor hunting activities.[45] Two other cases were subsequently identified in Florida, and a fourth case of anthrax—via cutaneous exposure—was identified in a female employee at NBC News in New York City.[43] Investigators then realized that the exposures resulted from anthrax-containing letters placed in the mail. On October 15, Senate Majority Leader Tom Daschle's office received

a letter that threatened an anthrax attack and also contained anthrax spores. The Hart Senate Office Building in Washington, DC, was subsequently closed.[46] By the end of the year, anthrax-laden letters placed in the mail had caused 22 cases of anthrax-related illness (11 inhalational [all confirmed], and 11 cutaneous anthrax [seven confirmed, four suspected]) and five deaths. Almost all anthrax cases were among postal workers and those who had handled mail.[47,48] For two cases, it was difficult to determine exact exposure risk. A 12th cutaneous anthrax case related to these mailings occurred in March 2002 in a Texas laboratory where anthrax samples had been processed.[49,50]

Case Review of 2001 Anthrax Mailings
Biological Agents: *B anthracis*, gram-positive bacillus
Potential Epidemiological Clues: 3, 5, 9, 11

Review: An unprecedented national response occurred involving thousands of investigators from federal, state, and local agencies. Close collaboration was required of all agencies, and the CDC and FBI formed partnerships to conduct public health and criminal investigations.[9]

Public health surveillance to detect previously unreported anthrax cases and determine that no new cases were taking place severely strained public health capacity.[51,52] This outbreak highlighted the importance of containing not only the disease but also public panic.

The Laboratory Response Network, a multilevel network connecting local and state public health laboratories with national public health and military laboratories,[53] served as a lead resource for both identifying and ruling out a potential biological attack.[54] Molecular subtyping of *B anthracis* strains played an important role in the differentiation and identification of *B anthracis*. High-resolution molecular subtyping determined that the anthrax mail-related isolates were indistinguishable and likely came from a single source.[55]

Postal workers and others handling mail were shown to be at risk from the anthrax-containing letters[56] and contaminated postal machinery[57]; therefore, federal and state health officials instituted environmental sampling,[58] cleaning,[59] and protective measures as well as antibiotic prophylaxis.[60] Similar protective actions were taken after discovery of the anthrax spore-laden envelope opened in the Senate Office Building.[45] It was later determined that patients frequently did not complete the recommended prophylaxis duration.[61]

As a direct result of the anthrax mailings, on January 31, 2002, the federal government made $1.1 billion available to the states for BT preparedness.[62] Disease detection and notification efforts, a cornerstone of BT preparedness, have changed dramatically since the incident. Continuing efforts to strengthen the public health workforce should help to better detect, respond, and manage a future BT crisis.[63]

Lessons Learned: An enhanced index of suspicion is necessary for unusual manifestations of BT diseases. Healthcare providers can learn to heighten their index of suspicion and diagnosis early if information is available and they are aware of a disease in a community.

Fine particles of a biological agent can become airborne, thereby contaminating areas and placing persons at risk without direct exposure to the contaminated vehicle. An exposure can occur anywhere along the path of the contaminant, and increased medical surveillance and possibly prophylaxis should be instituted for anyone with potential pathogen exposure.

Risk communication and key messages are important to contain potential public unrest.

Ricin—South Carolina and Washington, DC, 2003–2004

After a terrorist plot to use ricin in England in January 2003,[64] this plant-based toxin (a ribosome-inactivating protein) was found in a South Carolina postal facility in October 2003.[65] Ricin was also discovered in the office of Senator Bill Frist at the Dirksen Senate Office Building in Washington, DC, on February 3, 2004.[66]

On October 15, 2003, an envelope containing a note threatening to poison water supplies with ricin and a sealed container were processed at a mail-processing plant and distribution facility in Greenville, South Carolina. Laboratory testing at the CDC on October 21 confirmed the presence of ricin in the container. State health authorities interviewed all postal workers at the facility, and statewide surveillance for illness consistent with ricin exposure was initiated. The postal facility was closed on October 22, and epidemiological and environmental investigations were conducted. Hospital emergency departments, clinicians, health departments, and the postal facility were asked to report any cases consistent with ricin exposure. State poison control center and intensive care unit charts at seven hospitals near the postal facility were reviewed daily. A medical toxicologist and epidemiologists interviewed all 36 workers at the postal facility to determine whether any were ill, and no postal employees had illness indicating ricin exposure. CDC also conducted environmental testing at the postal facility; all tests were subsequently found negative for ricin.[65]

In 2013 ricin poisoning again became a newsworthy event when ricin-laced letters were sent to President Barack Obama, New York City Mayor Michael Bloomberg, and a gun control lobbyist in Washington, DC. A Texas woman, Shannon Guess Richardson, was arrested and charged in this case, after her confession that she had mailed the letters, and left incriminating evidence that her husband had committed this biocrime.[67]

Case Review of 2003–2004 Ricin Events
Biological Agents: *Ricin communis* toxin
Potential Epidemiological Clues: 3, 11

Review: Ricin is a potent cytotoxin derived from the beans of the castor plant (*R communis*). Ricin will likely continue to be a threat agent because castor beans are grown and used commercially worldwide, and the toxin can be readily extracted.

Ricin is considered to be a more rapidly acting toxin when it is ingested or inhaled than when injected. Treatment for ricin toxicity is supportive care because no antidote exists, and the toxin cannot be removed by dialysis.

Difficulties inherent in responding to a threat of ricin use include the lack of a detection method for locating ricin in clinical samples. A mild ricin poisoning may resemble gastroenteritis or respiratory illness. Ingestion of higher ricin doses leads to severe gastrointestinal symptoms followed by vascular collapse and death; inhalation of a small particle aerosol may produce severe respiratory symptoms followed by acute hypoxic respiratory failure.[68]

Lessons Learned: Any ricin threat should be investigated. As no cases resulted from the above exposures, it is likely that the material used in these incidents was not processed, purified, or dispersed in a manner that would cause human illness.

Biological agents that are readily available in nature remain a threat.

Accidental Release of Biological Agents

The following case studies document the events that transpired after what is understood to be the accidental release of BW agents, *B anthracis*[16] and *Variola major*,[69] in the Soviet Union during the 1970s. The former Soviet Union had a massive state-sponsored biological weapons program, as documented by its former deputy director Ken Alibek in his book, *Biohazard*.[70] This account provides frightening emphasis on the dangers to innocent populations from purposeful biological weapon development.

Anthrax—Sverdlovsk, Soviet Union, 1979

In April and May 1979, the largest documented outbreak of human inhalational anthrax occurred in Sverdlovsk in the Soviet Union (now Ekaterinburg, Russia), with at least 77 cases of disease and 66 deaths. Soviet authorities initially reported the occurrence of a gastrointestinal anthrax outbreak. Gastrointestinal anthrax is an uncharacteristic clinical manifestation from ingesting *B anthracis* spores, although it occasionally occurs in the republics of the former Soviet Union.[16,71] When case history and autopsy results were reexamined by a joint team of Soviet and Western physicians and scientists, it became apparent that the Sverdlovsk outbreak and subsequent deaths had been caused by inhalational anthrax.[16] The geographic distribution of human cases coupled with the location of animal cases indicated that all anthrax disease occurred within a very narrow geographic zone (4 km for the humans, 40 km for the animals) from a point of origin in Sverdlovsk. Historical meteorological data, when combined with this case distribution, demonstrated a point of origin at a military microbiological facility, Compound 19.[16] These data also indicated that the most likely day on which this event occurred was April 2, 1979.[16]

Public health authorities established an emergency commission that directed public health response measures on April 10, 1979, which did not include the Soviet military. A triage response was established at Sverdlovsk city hospital by April 12. Separate areas were designated for screening suspected cases and for treating nonsystemic cutaneous anthrax cases and for intensive care and autopsy. Anthrax illness was not believed to be be transmitted from person-to-person. Those who had died were placed in coffins containing chlorinated lime and buried in a separate part of the city cemetery. Hospital and factory workers were recruited into teams that visited homes of both suspected and confirmed cases throughout the city to conduct medical interviews, dispense tetracycline as a prophylactic antibiotic, disinfect kitchens and patient sickrooms, and collect meat and environmental samples for microbiological testing. Local fire brigades washed trees and building exteriors in the section of the city where most cases were located. Some of the control measures that authorities enacted likely had little value. Stray dogs were shot, and some unpaved streets were paved. Newspaper articles were published, and posters were displayed that warned residents of the anthrax risk from eating uninspected meat or having contact with sick animals. Meat shipments entering the city were examined, and uninspected meat was embargoed and burned. In mid-April a voluntary anthrax vaccination program for healthy individuals aged 18 to 55 years was begun in the part of the city where most of the infected persons lived. Of the 59,000 people eligible to receive anthrax vaccine, about 80% received at least a single dose of the vaccine.[16,72]

Case Review of 1979 Sverdlovsk Anthrax Release
Biological Agents: *B anthracis*, gram-positive bacillus
Potential Epidemiological Clues: 1, 2, 3, 4, 7, 9, 10

Review: In the absence of confirmatory information of an aerosol anthrax release, the public health response was spectacular. Research has estimated that approximately 14% more deaths would have occurred in Sverdlovsk in the absence of the public health intervention that included distribution of antibiotics and vaccination.[72]

The Soviet military's secrecy hid many facts that would have helped physicians to diagnose and treat inhalational anthrax exposure. It is possible that many more individuals

than existing medical records indicate may have become ill and recovered, or died.[73] Ambulance personnel often made an initial case diagnosis of pneumonia.[74]

Government authorities confiscated patient records and autopsy reports from the hospital. Some of these records could have provided invaluable inhalational anthrax medical intervention information from those patients that survived. Along with the absence of an epidemiological investigation at Sverdlovsk, this was a stunning loss of vital information for BW defense purposes.[75]

Former Soviet physicians released important information about anthrax prophylaxis and treatment, some of who took tissue samples and records home at their own considerable personal risk. This information indicated that the incubation period for inhalational anthrax may be as long as 2 months and that an antibiotic course of 5 days likely prolonged the incubation period for illness.[75]

Molecular analysis of tissue samples collected from 11 victims, and retained by Sverdlovsk physicians, indicate that these cases had been exposed to a number of different *B anthracis* strains.[76]

Lessons Learned: Retrospective pathology findings from victims, weather patterns, and geographic mapping can help to determine the outbreak source and also whether it spread.

Public health personnel in Sverdlovsk instituted effective preventive measures before they knew exactly what the exposure was or the cause of the illnesses, and they used information from cases to determine possible exposure routes.

Once the disease agent was determined, prophylactic antibiotics and vaccination and protective environmental measures could be provided.

Studies of Natural Outbreaks for Potential Bioweapon Use

Although the following accounts are examples of naturally occurring outbreaks, some components raise suspicion that they were intentionally caused. Subsequent to the 1999 WNV outbreak in New York City, suggestions were made that Iraqi operatives could have covertly released a biological weapon. These allegations by Richard Preston in the *New Yorker* magazine were based on documentation showing that CDC had provided Iraq with various biological agents from 1984 through 1993, including *Y pestis*, dengue, and WNV,[77,78] together with the fact that the Iraqi government was known to have had a covert biological weapons program.[79] Although never shown to be anything other than an imported disease outbreak occurring in an opportunistic manner, this claim received a lot of political attention. Similar allegations of the covert use of a biological weapon could have been made with other outbreaks, including the 2000 Martha's Vineyard (Massachusetts) tularemia outbreak, and they were made during the 1999 through 2000 Kosovo tularemia outbreak, which occurred during wartime.

West Nile Virus, New York, New York, 1999

An outbreak of an unusual encephalitis was first recognized in New York City in late August 1999. On August 23 an infectious disease physician from a Queens hospital contacted the New York City Department of Hygiene and Mental Health to report two patients with encephalitis. The health department then conducted a citywide investigation that revealed a cluster of six patients with encephalitis in which five had profound muscle weakness and four required respiratory support. CDC's initial clinical tests of these patients' cerebrospinal fluid and serum samples indicated positive results for Saint Louis encephalitis on September 3. More cases of encephalitis in New York City ensued, and because eight of the earliest cases were residents of a 2-square-mile area in Queens, aerial and ground applications of mosquito pesticides began in northern Queens and South Bronx on September 3.[80]

Active encephalitis surveillance began in New York City on August 30 and in nearby Nassau and Westchester counties on September 3. A clinical case was defined as a presumptive diagnosis of viral encephalitis with or without muscle weakness or acute flaccid paralysis, Guillain-Barré syndrome, aseptic meningitis, or presence of the clinical syndrome as identified in earlier cases.[80] Before and during this outbreak, an observed increase in bird deaths (especially crows) was noted in New York City.[14] The USDA National Veterinary Services Laboratory in Ames, Iowa, analyzed tissue specimens taken from dead birds in the Bronx Zoo for common avian pathogens and equine encephalitis. When these test results were negative, the samples were forwarded to CDC, which revealed on September 23 that the virus was similar to WNV in genetic composition.[81] At that time WNV had never been isolated in the western hemisphere.

Concurrently, brain tissue from three New York City encephalitis case deaths tested positive for WNV at the University of California at Irvine. As of September 28, 17 confirmed and 20 probable cases had occurred in New York City and Nassau and Westchester counties, resulting in four deaths. Onset dates were from August 5 through September 16. The median age of the patients was 71 years (range 15–87 years). By October 5 the number of laboratory-positive cases had increased to 50 (27 confirmed and 23 probable). Emergency telephone hotlines were established in New York City on September 3, and 130,000 calls were received by September 28. About 300,000 cans of *N,N*-diethylmetatoluamide (DEET)-based mosquito repellant were distributed citywide through local firehouses, and 750,000 public health leaflets were distributed with information on protection from mosquito bites. Radio, television, and the Internet provided public health messages.[80]

A seroprevalence survey later determined that approximately 100 asymptomatic infections and 30 WNV fever cases occurred for each WNV encephalitis case previously identified in the New York City area.[82]

Case Review of 1999 West Nile Virus Cases
Biological Agents: WNV, a flavivirus
Potential Epidemiological Clues: 1, 2, 3, 7
Review: Although some suggestions were made that this could have been a bioterrorist attack, the appearance of WNV in New York City in 1999 and its subsequent spread to the rest of the United States was most likely a natural occurrence.

Saint Louis encephalitis and WNV are antigenically related, and cross reactions can occur with some serologic testing.[80] Limitations of serologic testing underscore the importance of isolation and identification of virus.[80]

Within its normal geographic area of distribution in Africa, West Asia, and the Middle East, birds do not normally show symptoms when infected with WNV.[83] WNV from this part of the world occasionally causes epidemics in Europe that may be initiated by migrant birds.[84,85] An epizootic that results in the deaths of large numbers of crows may be a clue that either a new population is susceptible to the virus or a new, more virulent strain of a virus has been introduced.[80]

WNV is transmitted primarily by Culex mosquitoes,[86] which contributed to its spread in the United States after the 1999 outbreak.[87]

Genetic testing revealed that the virus was 99% identical to a virus isolated in 1999 from a goose in Israel.[88] Potential routes for WNV introduction include importation of WNV-infected birds, mosquitoes, or ill persons. The New York City area where WNV was prevalent includes two large international airports.[89]

Before this outbreak, death was rarely associated with WNV infection.[90] In patients with WNV encephalitis, computer-assisted tomography often revealed preexisting lesions and chronic changes in brain tissue,[91] perhaps suggestive of the potential for a greater susceptibility to deleterious outcome in elderly persons.

Lessons Learned: This outbreak emphasizes the important relationship among veterinarians, physicians, and public health authorities in disease surveillance, and the importance of considering uncommon pathogens.[90]

The incident is an example of a typical zoonotic disease epidemic pattern—a natural epidemic occurred first among birds, followed by disease in humans.

The origin of outbreaks fitting some of the clues for a biological attack (a new disease for a geographic region) cannot be immediately determined without further investigation. Emerging diseases, whether new for a particular geographic area, like WNV, or a totally new disease (eg, severe acute respiratory syndrome or Middle East Respiratory Syndrome coronavirus), are not uncommon.

Tularemia, Martha's Vineyard, Massachusetts, 2000

During the summer of 2000, an outbreak of primary pneumonic tularemia occurred on Martha's Vineyard, Massachusetts.[92] In July five cases of primary pneumonic tularemia were reported, with onset dates between May 30 and June 22. The Massachusetts Department of Public Health and CDC initiated active surveillance, and 15 confirmed tularemia cases were subsequently identified. A confirmed case was defined as occurring in a visitor or resident to Martha's Vineyard who had symptoms suggesting primary pneumonic tularemia; was ill between May 15 and October 31, 2000; and had test results showing a serum titer of anti-*F tularensis* antibody of at least 1:128 on an agglutination assay. Of these cases, 11 had the pneumonic form of the disease, two had ulceroglandular disease, and two had fever and malaise. Fourteen of the patients were male, and the median age was 43 years (range 13–59). One 43-year-old man died of primary pneumonic tularemia.[92]

Control subjects for a case-control study were obtained by random-digit dialing to Martha's Vineyard residents, enrolling 100 control subjects at least 18 years old that had spent at least 15 days on the island between May 15 and their September interviews. Both ill persons and control subjects were questioned about occupation, landscaping activities, animal and arthropod exposures, recreational and outdoor activities, and general health history and status. Information was obtained about exposure to risk factors between May 15 and the interview, and for 2 weeks before illness for ill persons and 2 weeks before interview for control subjects.[92]

The suspected site of exposure for each patient was visited. Activities that may have led to exposure (eg, lawn mowing and "weed whacking") were reproduced, and environmental and personal air samples were taken. Samples from soil, water, grass, wild mammals, and dogs were also taken. Epidemiological analysis revealed that in the 2 weeks before illness, using a lawn mower or brush cutter was significantly associated with illness. Of all the environmental and animal tissue samples taken, only two were positive for *F tularensis*: (1) a striped skunk and (2) a Norway rat.[92]

Case Review of 2000 Martha's Vineyard Tularemia Outbreak
Biological Agents: *F tularensis*, a gram-negative bacillus
Potential Epidemiological Clues: 1, 2, 3, 9
Review: Caused by a gram-negative bacillus *F tularensis*, tularemia is a rare infection in the United States. Between 2001 and 2010, a median number of 126.5 cases per year (range: 90–154 cases per year) was reported.[91] More than half of all cases reported during these 11 years came from Arkansas, Missouri, South Dakota, and Oklahoma, and most cases were acquired from tick bites or contact with infected rabbits. Higher incidences of the disease have been noted in persons ages 5 to 9 and older than 75, and incidence was greatest among Native Americans and Alaskan natives.[93]

The only other previously reported pneumonic tularemia outbreak in the United States had occurred on Martha's Vineyard during the summer of 1978.[94] During a single week (July 30–August 6) seven persons stayed in a vacation cottage. By August 12, six of these had a fever, headache, and myalgia; and the seventh had a low-grade fever by August 19. A search for additional cases on the island uncovered six other tularemia cases, five of which were pneumonic, and one was ulceroglandular. No source for the disease exposure was discovered, although two rabbits later found dead were culture-positive for *F tularensis*.

Tularemia had been reported sporadically since rabbits had been introduced to Martha's Vineyard in the 1930s,[93] and pneumonic tularemia was first reported in Massachusetts in 1947.[95] Classic research on human tularemia rates showed that very high rabbit populations increase the tularemia hazard.[96]

Hospital clinicians on Martha's Vineyard initially detected this outbreak and recognized tularemia caused pneumonic summer illness,[97] in part based on the experiences with the previous outbreak.[94]

Feldman et al proposed in this outbreak *F tularensis* was shed in animal excreta, persisted in the environment, and infected persons after mechanical aerosolization and inhalation. This is a likely exposure scenario, given the principal form of primary pneumonic tularemia seen in these cases and strong epidemiological association with grass cutting.[92]

A seroprevalence survey conducted in 2001 in Martha's Vineyard demonstrated that landscapers were more likely to have an antibody titer to *F tularensis* than nonlandscapers, revealing an occupational risk for tularemia.[92]

Lessons Learned: Naturally occurring disease can present in the pneumonic form. However, if tularemia were used as a biological weapon, an aerosolized release would probably result in multiple simultaneous cases presenting with the pneumonic form of the disease.[97]

There may also be disease transmission mechanisms (in this example, grass cutting) that are unknown or poorly understood.[98]

Tularemia, Kosovo, 1999–2000

After a decade of political crises and warfare, a large outbreak of tularemia occurred in Kosovo from 1999 through 2000. Tularemia had not been reported in Kosovo since 1974.[99] By April 2000, 250 suspected cases had been identified and spread nationwide, but most cases existed in the western area where ethnic Albanians resided.[100]

Unusual outbreaks of zoonoses or vectorborne disease may readily occur in war-torn or crisis-afflicted regions that have previously been free of these diseases. Historically, outbreaks of typhus, plague, cholera, dysentery, typhoid fever, and smallpox have long been observed in war-torn regions.[101] Among the earliest historic examples is the plague of Athens that arose during the second year of the Peloponnesian War, as described by Thucydides.[102] Speculation may arise that these epidemics were purposefully caused. Many biological agents are zoonotic pathogens,[99] including tularemia, a category A BW pathogen. Purposeful use of this pathogen merits consideration when such an outbreak occurs with a pathogen having the potential to be a biological weapon.[103]

Remarks made by the head epidemiologist at the Kosovo Institute of Public Health about unidentifiable ampoules and white powders discovered near various wells could not be verified and added to a perception of use of a biological weapon by Serbian forces.[99] *F tularensis biovar tularensis* (type A) is highly pathogenic for humans. It is found mostly in North America and has been developed for use as a biological weapon. Disease progression often follows an acute and severe course, with prominent pneumonitis. *F tularensis biovar holarctica* (type B) is less pathogenic and is found throughout the northern hemisphere.[104] To further complicate matters, a 1998 report documented that type A tularemia had been introduced into arthropod populations in the nearby Slovak Republic.[105] The United Nations mission in Kosovo requested that the World Health Organization assist Kosovar health authorities in an epidemiological investigation of the tularemia outbreak. Teams of international and Kosovar public health personnel collaborated in epidemiological, environmental, and microbiological field and laboratory investigations.[106]

Tularemia cases were discovered by both prospective surveillance and retrospective hospital review of a pharyngitis and cervical lymphadenitis syndrome. Ill persons were clinically examined and interviewed, blood samples were taken from suspected cases, and antibiotics were prescribed as appropriate. Rural villagers reported an increase in mice and rats in the summer of 1999. A causal association was suspected between the increased population density of rodents and human tularemia cases. Tularemia is naturally transmitted to humans via small lesions in the skin of persons handling diseased rabbits, ingestion of contaminated water or food, bites of infectious arthropods, or inhalation of infective dusts.[99]

A matched case-control study was conducted with paired households in villages in regions with the greatest number of reported cases. Case households had one or more family members with a laboratory-confirmed case of tularemia as of November 1, 1999. Control households were the two households closest to a suspected case household, having no individuals with the disease, and the person who prepared the family's food was serologically negative for tularemia. Blood specimens were also drawn from all suspected cases. Questionnaires were completed on household

food consumption, water supply, presence of rodents, and condition of wells and food preparation and storage areas. The study period began a month before symptom onset of the first case in the suspected case household. Well water sampling and rodent collection and analysis were performed.

By June 30, 2000, more than 900 suspected tularemia cases had been discovered. From these, 327 were confirmed as serologically positive. The earliest onset of reported symptoms in the confirmed cases was October 1999, with an epidemic peak in January 2000. Confirmed cases were identified in 21 of 29 Kosovo municipalities. Cases were equally distributed by sex, and all age groups were equally affected. Case households were more likely to have nonrodent-proof water sources, and members in these households were less likely to have eaten fresh vegetables. Risk factors for case households included rodent feces in food preparation and storage areas and large numbers of field mice observed outside the house. Of the field samples collected, positive antigen for *F tularensis* was detected in striped field mouse and black rat fecal specimens.

Case Review of 2000 Kosovo Tularemia Outbreak
Biological Agents: *F tularensis*, a gram-negative bacillus
Potential Epidemiological Clues: 1, 3, 5, 9
Review: Clinical and serologic evidence indicate that a tularemia outbreak occurred in Kosovo from October 1999 through May 2000. The case-control study indicated that transmission of tularemia was foodborne based on the associations of illness and large numbers of rodents in the household environment, rodent contamination of food storage and preparation areas, and consumption of certain uncooked foods. Unprotected water that was not boiled likely contributed to the outbreak.

Initial field investigations rapidly demonstrated that a widespread natural event was occurring and likely resulted from the unusual environmental conditions existing in war-torn Kosovo. The principal populations affected by the tularemia outbreak were ethnic Albanians in rural farming villages with limited economic resources. These people had fled during North Atlantic Treaty Organization bombing and Serbian reprisals during the spring of 1999. Refugees discovered bombed and ransacked homes, unprotected food storage areas, unharvested crops, damaged wells, and a rodent population explosion when they returned to their cottages. Both ignorance of infection and lack of hygienic measures contributed to a foodborne infection in the population.[99]

F tularensis can survive for prolonged periods in cold, moist conditions.

A natural decrease in rodent population resulting from the cold winter, food shortages, and the disease itself likely all helped to end the zoonoses.[99]

Although tularemia was not recognized endemically or enzootically in Kosovo before the 1999 through 2000 outbreak, it became well established in a host reservoir. A second outbreak occurred there in 2003, causing more than 300 cases of oropharyngeal tularemia.[107]

Historically, war in Europe caused tularemia outbreaks. During World War II, an outbreak of more than 100,000 cases of tularemia occurred in the Soviet Union,[108] and outbreaks with hundreds of cases following the war occurred in Austria and France.[107]

Lessons Learned: War provides a fertile ground for the reemergence of diseases and potential cover for BW agent use that is plausible and may go unrecognized as a BW event. An extensive epidemiological investigation must be conducted to conclude or disprove that a BW event has occurred.

Q Fever, Iraq 2005

Q fever is a zoonotic disease caused by *C burnetii*, a bacteria found worldwide. Human cases occur from inhalation of aerosols or windborne dust contaminated with *C burnetii* from birth products, milk, urine, and feces of infected animals—most frequently cattle, camel, goats, and sheep. Infections can also occur from ingesting raw milk or eggs as well as tick bites or human-to-human transmission.[109] Due to the bacteria's ability to survive in harsh environmental climates and its high infectivity, there is concern of its use as a biological weapon. The United States developed Q fever as a biological weapon before ratifying the Biological Weapons Convention. The CDC classifies *C burnetii* as a Category B agent.

From June 18 to July 10, 2005, 22 of 38 Marines (58%) from a single platoon in Al Asad, Iraq, experienced a febrile illness.[110] All patients had a rapid onset of fever and chills, and the majority had headache, respiratory, and gastrointestinal symptoms. The patients were diagnosed with upper respiratory infection or atypical pneumonia because there was no diagnostic capability. Subsequent testing was negative for multiple respiratory pathogens. Follow-up serologic testing 6 weeks later on 9 of the affected patients revealed positive Q fever immunoglobulin for all 9, with 10 unaffected persons from the same unit negative for antibody.[110]

After confirmation of Q fever, the researchers distributed follow-up questionnaires to the company that included the affected platoon. They found an association between infection and exposure to ticks and a trend toward association with exposure to camels and the birth of both sheep and dogs. Although the authors did not have a sufficient sample size to confirm all risk factors, they hypothesize that this particular platoon may have sought shelter in an area that was heavily infected secondary to recent animal inhabitation and birthing or ticks.[110]

Before this outbreak, Q fever cases had been reported in US service members deployed to Iraq. An evaluation of 62 cases of pneumonia in 2003 found eight had seroconverted with Q fever antibody,[111] and an additional four diagnosed cases in 2003 and 2004 were reported.[112,113] Three cases of Q fever occurred in US forces in Iraq during the first Persian Gulf War (1990–1991).[114] Since the 2005 outbreak in the Marines, more cases have been reported, and two serosurveys have been performed. One serosurvey revealed 10% of 909 military personnel hospitalized during deployment in 2003–2004 with symptoms compatible with Q fever seroconverted,[115] and another serosurvey studying the same company affected in the outbreak in 2005 found seroconversion in 7.2% of 279 tested.[116] The British military has also published occurrences of Q fever in deployed forces, including 26% of "Helmand Fever" cases caused by Q fever in Afghanistan.[117]

Surveillance of deployed military working dogs in Iraq revealed no seroconversions in 2007–2008, compared to a 5.5% seroconversion in feral dogs.[118] This lack of infection is probably secondary to tick control and doxycycline prophylaxis for the military working dogs.

Case Review of 2005 Q fever cases

Biological Agent: *C burnetii*, gram-negative, facultative, intracellular coccobacillus

Potential Epidemiological Clues: 1, 4

Review: An attack rate of 58% occurred in one platoon. Although the research team was unable to determine exact movements of the platoon, it is likely they had an exposure different from the other platoons.

A relatively short epidemic curve, especially with a long and variable incubation period for the pathogen, suggests a point source. This outbreak probably resulted from an isolated exposure over a short time period.

It is a disease of relatively high severity, had an unknown cause at time of outbreak, and can raise concern about potential intentional cause.

Q fever is considered a potential bioweapon and a cause for concern.

Lessons Learned: All medical personnel should know what diseases are endemic in the area and previous history in deployed forces.

Cases should be reported immediately to allow dissemination of recommended diagnostics and treatment. In this case, the Armed Forces Infectious Disease Society published a set of practice guidelines for diagnosis and management of Q fever to assist deployed medical personnel.[119]

Investigate outbreaks of disease, even after resolution. Knowledge obtained will assist in preventing, recognizing, and rapidly treating future cases.

EPIDEMIOLOGICAL ASSESSMENT TOOL

It is useful for public health authorities to determine whether an infectious disease outbreak is intentional. Grunow and Finke developed an epidemiological assessment tool to rule out biological agent use during infectious disease outbreaks.[98] This assessment tool's relevance was demonstrated by analysis of the 1999–2000 Kosovo tularemia outbreak.[99] In their evaluation scheme, each assessment criterion can be given a varying number of points dependent on its presence and characteristics. There are two types of evaluation criteria: (1) nonconclusive and (2) conclusive. The most significant nonconclusive criteria include a biological threat or risk, special aspects of a biological agent, a high concentration of biological agent in the environment, and epidemic characteristics. Conclusive criteria include the unquestionable identification of the cause of illness as a BW agent (eg, demonstrating modifications that make the agent different from its naturally occurring equivalent, such as stabilizers or physical modifications) or proof of the release of such an agent as a biological weapon. With conclusive criteria, additional confirmatory information is unnecessary.[99]

According to Grunow and Finke's nonconclusive criteria, a biological risk may be considered if a political or terrorist environment exists from which a biological attack could originate:

- **Biorisk**. Are BW agents available, with the means for distribution, and the will to use them? Or can an outbreak be explained by natural biological hazards, or the changes incurred by military conflict?
- **Biothreat**. Does a biological threat exist by virtue of a group having a BW agent and credibly threatening to use it?
- **Special aspects**. Is there plausible evidence of purposeful manipulation of a pathogen?
- **Geographic distribution**. Is the disease's geographic distribution likely given its locale? With the advent of a nonendemic pathogen, a thorough evaluation should include epidemiological, epizootic, ecological, microbiological, and forensic analysis.
- **Environmental concentration**. Is there a high environmental concentration of the pathogen?
- **Epidemic intensity**. Is the course of illness relative to disease intensity and spread in the population expected in naturally occurring illness?
- **Transmission mode**. Was the path of disease transmission considered naturally occurring? The appearance of a naturally occurring epidemic in itself does not rule out the purposeful use of a BW agent.

- **Time**. Was the seasonal timing of the epidemic unusual?
- **Unusually rapid spread**. Was the spread of the epidemic unusually rapid?
- **Population limitation**. Was the epidemic limited to a specific (target) population? If certain persons were given prior warning of a BW attack, then they may protect themselves, as compared to naïve target populations.
- **Clinical**. Were the clinical manifestations of the disease to be expected?

The Grunow-Finke epidemiological assessment procedure (Table 2-1) was used to evaluate the case studies presented in this chapter. To use the assessment tool uniformly for all the events described in this chapter, some artificial constraints were placed on the analysis. For this exercise, only nonconclusive criteria were used because the use of conclusive criteria may have excluded many of the case studies with a retrospective assessment. During an outbreak investigation, however, epidemiological investigators would also initially use the nonconclusive evaluation criteria. With

TABLE 2-1

EPIDEMIOLOGICAL ASSESSMENT AND EVALUATION OF CASE STUDY OUTBREAKS

Nonconclusive Criteria	Assessment (possible points)	Weighting Factor	Maximum No. of Points	1915 Anthrax Eastern USA	1971 Smallpox Aralsk	1979 Anthrax Sverdlovsk	1984 Salmonella Oregon	1995 Anthrax Tokyo	1996 Shigella Texas
Biorisk	0–3	2	6	4	4	4	6	6	0
Biothreat	0–3	3	9	0	0	0	0	6	0
Special aspects	0–3	3	9	6	6	6	3	0	6
Geographic distribution	0–3	1	3	3	3	3	2	3	2
Environmental concentration	0–3	2	6	6	0	6	0	6	0
Epidemic intensity	0–3	1	3	3	3	3	3	0	3
Transmission mode	0–3	2	6	6	2	6	4	0	0
Time	0–3	1	3	3	3	3	1	0	1
Unusually rapid spread	0–3	1	3	3	1	3	3	0	3
Population limitation	0–3	1	3	1	0	1	0	0	3
Clinical	0–3	1	3	3	3	3	0	0	1
Score			**54**	**38**	**25**	**38**	**22**	**21**	**19**

Nonconclusive Criteria	1999 WNV NYC	1999 Tularemia Kosovo	2000 Tularemia Martha's Vineyard	2001 Anthrax USA	2003 Ricin USA	2005 Q Fever
Biorisk	6	2	0	6	6	2
Biothreat	6	3	0	6	9	6
Special aspects	0	0	0	9	0	0
Geographic distribution	3	3	3	3	3	0
Environmental concentration	4	4	4	6	6	0
Epidemic intensity	3	3	3	3	0	1
Transmission mode	2	2	6	6	0	0
Time	1	0	3	3	0	0
Unusually rapid spread	3	1	3	3	0	1
Population limitation	0	0	2	3	0	3
Clinical	1	1	3	3	0	0
Score	**29**	**19**	**27**	**51**	**24**	**13**

NYC: New York City
USA: United States of America
WNV: West Nile Virus

the exception of the 2001 anthrax and 2003 ricin events, none of the outbreaks described had been positively identified as having been caused by a biological agent until sometime after the events had occurred.

Grunow and Finke provide the following cut-off scores for nonconclusive criteria with respect to the likelihood of biological weapon use:

- unlikely (0%–33% confidence): 0 to 17 points;
- doubtful (18%–35% confidence): 18 to 35 points;
- likely (67%–94% confidence): 36 to 50 points; and
- highly likely (95%–100% confidence): 51 to 54 points.

Based on this scoring, only the 2001 anthrax mailings would be considered as highly likely to have been caused by a BW agent. The 1915 and 1979 anthrax events qualify as likely to have been caused by a BW agent. All of the other case study scenarios are either doubtful or unlikely to have been caused by a BW agent.

The authors conducted this evaluative exercise by consensus of opinion. Although subjective, the exercise underscores the challenges facing epidemiologists to determine whether a BT/BW event has occurred, unless direct evidence indicates a purposeful event, or someone credibly claims responsibility. The basic epidemiological principles described earlier in this chapter (including those needed for disease recognition) to determine the occurrence of an unnatural event, and for basic outbreak investigation, are the foundation of infectious disease response and control. Public health authorities must remain vigilant to quickly and appropriately respond to any infectious disease event.

IMPROVING RECOGNITION AND SURVEILLANCE OF BIOTERRORISM

Existing disease surveillance systems may not be sensitive enough to detect a few cases of illness, unless they are legally reportable diseases that have confirmed laboratory diagnoses. However, even before confirmed diagnoses, disease reporting can be initiated upon patient presentation to healthcare providers with initial diagnoses, laboratory testing, and the reason provided by the patient for the hospital visit. Clinicians, laboratories, hospitals, ancillary healthcare professionals, veterinarians, medical examiners, morticians, and others may be partners in reporting diseases to public health authorities.

If a medical surveillance system first detects a biological attack, there may already be a significant number of cases, and the available time to prevent further illness is short or perhaps already over. The point of release is the earliest detection point of a biological event. Some disease exposures could be prevented through publicized avoidance of the area at risk, prophylactic medication use, or vaccination of those exposed, coupled with immediate disease recognition and patient treatment. The Department of Homeland Security's BioWatch program has deployed biological detectors in major urban centers nationwide to detect trace amounts of airborne biological materials[120,121] to help determine the presence and geographic extent of a biological release to focus emergency public health response and consequence management. Such detectors could be of great utility when pre-positioned at large well-publicized gatherings or in cities that may be the greatest targets for terrorist activity.

Although deployed sensors may detect an agent's release, the infinite number of venues coupled with limited resources to position sensors and analyze air samples minimizes the chances that an agent release will be detected. In most instances, the earliest opportunity to detect an attack will be by recognizing ill patients. Depending on the agent, the mode of dissemination, and the number exposed, initial cases will present in different ways. If the disease is severe, such as is possible with category A biological agents, one properly diagnosed case will launch an investigation, as seen during the 2001 anthrax attacks.[47]

Even if the cause is initially unknown, extremely severe or rapidly fatal cases of illness in previously healthy individuals should be reported to public health authorities. If many people are exposed, as would be expected with a large aerosol release of a biological agent, an overwhelming number of people may eventually visit hospital emergency departments and outpatient clinics. Even with less severe disease, such cases should be recognized and quickly reported.

However, in the absence of confirmed laboratory diagnoses or high attack rates, infectious disease outbreaks are often not reported. If the disease is not rapidly fatal or cases are distributed among a variety of healthcare practitioners, it may not be readily apparent that a disease outbreak is under way. Therefore, there is a need for better awareness of the health of communities—a way to quickly detect shifts in potentially infectious diseases, whether of bioterrorist origin or not. This need has been recognized and has resulted in the proliferation of what are commonly known as syndromic surveillance systems.

Syndromic surveillance has been defined as the ongoing, systematic collection, analysis, and interpretation of data that precede diagnosis and can indicate a potential disease outbreak earlier than when public

health authorities would usually be notified.[122] The data used in syndromic surveillance systems are usually nonspecific potential signs and symptoms of an illness spectrum indicating that disease may be higher than expected in a community. These data can be from new or existing sources.[123] For syndrome surveillance of BT, the emphasis is on timeliness, with automated analysis and visualization tools such as Web-based graphs and maps. These tools provide information that initiates a public health investigation as soon as possible.[124]

Numerous regional and national syndromic surveillance systems have been developed, including programs that rely on data collected specifically for the surveillance system and those that use existing medical data (eg, diagnostic codes, chief complaints, nurse advice calls, ambulance runs) and other information (eg, pharmacy sales, absenteeism, calls to poison control centers, Internet searches for specific symptoms or pathogens, participatory epidemiology where people voluntarily provide information to a system like Flu Near You[125] or even scanning Twitter feeds and other social media sites for the use of terms related to illness) to detect changes in population health. Systems that use active data collection can be "drop-in" (those instituted for a specific high-threat time) such as those performed immediately after September 11, 2001,[126–128] or during large gatherings for sports (eg, the Olympics) or other events,[129] or they can be sustained systems for continuous surveillance.[69,130] Systems that require new data entry benefit from greater specificity in the type of syndromes and illnesses reported, but they require extra work and are difficult to maintain. Systems that use existing data can be less specific, especially with information taken from behaviors early in the disease, such as over-the-counter pharmacy sales, absenteeism, Internet searches, and social media use. However, these programs have the large advantage of continuous data streams that are not dependent on provider input or influenced by news reports of disease rates. Such systems (examples of which are described below) have become standard in many health departments, the military, and CDC.

In the US Department of Defense, the Electronic Surveillance System for the Early Notification of Community-based Epidemics uses outpatient diagnostic *International Classification of Diseases, Ninth Revision* codes, chief complaints, radiology and laboratory tests, and pharmacy prescriptions to track disease groups in military beneficiaries. Temporal and spatial data are presented through a Web-based interface, and statistical algorithms are run to detect any aberrations that could indicate a disease outbreak.[131] This system is available for all permanent US military treatment facilities worldwide. Some local and state health departments use civilian versions of the Electronic Surveillance System for the Early Notification of Community-based Epidemics. Other civilian systems, such as the North Carolina Disease Event Tracking and Epidemiologic Collection Tool[132] and various software packages made available by the Real-time Outbreak and Disease Surveillance Laboratory at the Department of Biomedical Informatics at the University of Pittsburgh,[133] and the EpiCenter application[134] also use syndromic information from emergency departments, 911 calls, ambulance runs, and poison control center calls to monitor the health of populations.

CDC has developed the BioSense 2.0 program using national data sources such as the US Department of Defense and Department of Veterans Affairs outpatient diagnostic codes, state and local emergency department visits, and laboratory test orders from commercial vendors to track disease patterns nationwide. The information is provided in a Web-based format to health departments.[135] Algorithms are run on the data and send out an alert when levels of medical visits or laboratory test orders exceed those expected. The information is presented in temporal and spatial format, allowing the health department to track disease based on the patient's home zip code. The BioSense 2.0 goal is to facilitate sharing of automated detection and visualization algorithms and promote national standards.

Despite the proliferation of systems, there are definite limitations in the ability to detect bioterrorist attacks using syndromic surveillance. Some have argued that even if syndromic surveillance could detect an outbreak faster than traditional methods, the advanced warning may not assist with disease mitigation.[73] The warning may not be early enough or effective countermeasures may not be available. In addition, although nonspecific data such as absenteeism and social media may provide some early warning, it is very difficult to institute preventive measures without more specific information. However, nonspecific data can still serve as an early indicator, prompting authorities to monitor specific data sources more carefully.

Most importantly, because a BT attack can present in a variety of ways depending on the agent, population, method of dispersal, and environment, it is impossible to predict how any individual surveillance system will perform. It is generally agreed that most syndromic surveillance systems will not detect a few cases of disease, but they can assist in detecting more widespread disease increases and assessing the population impact, an outbreak's spread, and the success of mitigation efforts. The coverage area of the surveillance system is crucial in determining outbreak detection sensitivity in any part of a community. In the future, syndromic surveillance will probably be based on national models

such as BioSense 2.0 and use readily available electronic databases. Local health departments could then build on a national system using local data that can improve population coverage. Future disease monitoring and reporting systems need to be seamlessly integrated with other traditional disease surveillance systems. Ideally, these systems should also help to educate clinicians on the importance of maintaining a high index of suspicion and to promptly report unusual diseases or disease clusters to public health authorities.

POTENTIAL IMPACT OF ADVANCED MOLECULAR TECHNIQUES ON THE EPIDEMIOLOGY OF BIOWARFARE AND BIOTERRORISM

In addition to the use and application of syndromic surveillance for the detection of shifts in potentially infectious diseases, advances in technologies used for both disease diagnosis and surveillance are helping scientists and healthcare and public health professionals more quickly determine what is causing or has the potential to cause illness.[136–139] These technological advances, which include multiplex polymerase chain reaction, immunoassays, arrays, and even next-generation sequencing, allow a more accurate determination of not only the pathogen,[138,139–142] but also the presence of mutations or other factors that distinguish the organism(s) from previous outbreaks or near neighbors[143] and have the potential to result in more severe disease. These techniques have identified several emerging infectious diseases.[144–146]

Many of the technologies listed have been available for 30 years or more[147,148]; however, the increased speed and multiplex capability, lower cost, and greater application of the technologies as surveillance tools, combined with enhanced surveillance reporting systems, create a more likely environment for the detection of a possible natural or intentional biological event.[149–150] Specifically, the more routine use of sequencing has significantly affected biological sciences and has the potential to be influential in the arena of the epidemiology of biowarfare. Ten years ago the cost and sample-to-result time of sequencing were prohibitive for routine use. However, the cost and processing time continues to decrease, making the accessibility to sequencing more universal and easily adaptable for inclusion in pathogen identification and characterization.[151] In 1990 the National Institutes of Health and Department of Energy initiated the human genome project, which required 10 years to publish a working draft and cost millions of dollars.[152,153] A viral or bacterial genome can be sequenced in a few hours and can cost as little as $100 per isolate.[151,153–157] The use of sequence technology has been instrumental in not only pathogen detection and characterization, including mutations that increase morbidity and mortality, but also in the development of detection and diagnostic assays and therapeutic and prophylactic solutions and/or countermeasures.[155]

Most recently, sequencing was used in the Middle East Respiratory Syndrome coronavirus outbreak to identify the source of the disease, determine the distinction from severe acute respiratory syndrome corona virus,[143] and develop polymerase chain reaction detection and diagnostic capabilities.[23] Sequencing was also used in the H7N9 and H1N1 influenza outbreaks,[158,159] and in the *Escherichia coli* O104:H4 in Germany in 2011[160–162] to assist with identifying the causative agent and developing possible countermeasures. Although it appears as though these events have all been naturally occurring, the addition of characterization information in the form of sequence has allowed researchers to go back and look for possible index cases and the source or reservoir for the outbreak in humans. Rapid sequencing may also facilitate a more rapid vaccine development, as demonstrated in the use of novel techniques for influenza vaccine production.[163] The use of sequencing will continue to assist scientists and public health professionals in their search for not only the reservoir, point of exposure, possible nefarious intention, and comparison with currently known and well characterized diseases, but will also assist in limiting the spread of the disease and possible prevention of future outbreaks by identifying potential zoonotic crossover before it even occurs.[164–167]

Many organizations are conducting surveillance globally with the goal of predicting and preventing the next outbreak or pandemic, often in zoonotic sources.[164,165] The US Agency for International Development,[168] the US Department of Defense, and both for-profit and nonprofit, nongovernmental organizations are all engaged in surveillance efforts using some of these technological advancements to identify the next potential source of an outbreak and develop detection and prophylactic or therapeutic solutions and other nonmedical countermeasures to prevent such an event, or at the very least, to be well prepared to respond robustly and quickly.

However, not all uses of advanced technologies have been without controversy. One recent example of the use of sequencing in the creation of a potential BW agent came in late 2011 and continues today.[169–171] Flu researchers Ron Fouchier, of the Erasmus Medical

Center in The Netherlands, and Yoshihiro Kawaoka, of the University of Wisconsin-Madison, engineered more transmissible strains of H5N1, and, more recently, have focused on H7N9.[172,173] They believe genetic engineering can be used to determine which—if any—mutations accelerate the spread of influenza between mammals.[173–175] Additionally, scientists claim genetically modifying the H7N9 virus in the lab will help drive efforts to develop pandemic drugs and vaccines, and result in better preparedness and response.[175] However, not all scientists agree with the type of research being conducted, including infectious disease specialist Adel A F Mahmoud, of Princeton University.[171] Some scientists worry that these strains could escape the laboratory and possibly kill millions, or get in the hands of the wrong people.[173] Even the US National Science Advisory Board for Biosecurity became involved in this debate and has issued several rulings and restrictions on publication of information from this type of research. Dual use research considerations are also being carefully evaluated in some of these instances to ensure that the global populace is protected from potential harm.

There are other limitations with this type of information gathering and sharing. As seen during the *E coli* outbreak in Germany, when an initial error is made in the suspected source of the outbreak (in this case erroneously stated to be from Spanish cucumbers)[176] the information can seriously and detrimentally affect a nation, manufacturing or processing group, or product identified as the source.[177] Although the initial source of the outbreak was suspected based on epidemiological investigation and early molecular testing, the desire to release the information superseded molecular validation of the suspected outbreak source information[178]; it was not until the results obtained using advanced molecular techniques[160,179,180] combined with further epidemiological investigation identified the more likely outbreak source.[175,181] Additionally, some nations may not approve the release of information regarding an outbreak or may not allow other scientists to continue surveillance or investigations into the source if they feel their economy or other factors such as national security may be threatened. The existence, or lack thereof, of surveillance efforts, systems, and software solutions may also hinder the transfer of information regarding a potential outbreak or emerging infectious disease.[150]

The use of high throughput screening and sequencing technologies can also be instrumental in detection of anomalies indicative of not only natural mutation and resistance, but also engineered and intentional activities.[180,181] The addition of virulence factors such as plasmids that are not typical to given organisms, but convey greater morbidity, communicability, and so forth can be a potential sign of human manipulation. Phylogenetic comparison with known pathogens can not only narrow prevention and treatment options, but also can highlight a possible unnatural combination of strains. Sequence information can even be used to generate a pathogen of interest de-novo, without the pathogenic element, allowing for possible manipulation of once pathogenic organisms in a lower class safety environment and additional options for assay and countermeasure development.[182–184] However, this capability also allows for generation of dangerous pathogens with the proper authorization.[185] Although the knowledge obtained from sequencing can be very beneficial, it has the potential to cause harm if it falls into the wrong hands or is not accurate and does not get reported to the appropriate public health professionals.

However, as evidenced in the last few years when several anthrax and plague cases were detected in patients in the United States, advanced technologies can rapidly assist an epidemiologic investigation. Public health and laboratory officials moved quickly to investigate and determine the source of these infections; and using a combination of molecular techniques and epidemiological outbreak investigation, they found none were suspected to be intentionally caused.[186–193] The addition of advanced molecular techniques can lead to faster diagnosis, treatment, and determination of intent or origin of infection(s).

SUMMARY

Because management of BT and BW events depends on the disease surveillance, laboratory, and outbreak investigation capabilities of public health authorities, the science of epidemiology will always be the foundation for a response to these events. An enhanced index of suspicion, awareness of potential red flags, open lines of communication between local healthcare providers and law enforcement authorities, knowledge of historical outbreak investigation information, robust disease surveillance systems, and the use of advanced molecular techniques will improve the ability to respond to any future BT or BW event.

REFERENCES

1. The Greek Translation Portal. www.translatum.gr. Accessed January 14, 2014.

2. Beaglehole R, Bonita R, Kjellstrom T, eds. Basic epidemiology. In: *Communicable Disease Epidemiology*. Geneva, Switzerland: World Health Organization; 1993: Chap 7.

3. Mann JM, Martone WJ, Boyce JM, Kaufmann AF, Barnes AM, Weber NS. Endemic human plague in New Mexico: risk factors associated with infection. *J Infect Dis*. 1979;140:397–401.

4. Radovanovic Z, Djordjevic Z. Mass vaccination against smallpox and mortality in Yugoslavia in 1972. *Trans R Soc Trop Med Hyg*. 1979;73:122.

5. Meltzer MI, Damon I, LeDuc JW, Miller JD. Modeling potential responses to smallpox as a bioterrorist weapon. *Emerg Infect Dis*. 2001;7:959–969.

6. Milton DK. What was the primary mode of smallpox transmission? Implications for biodefense. *Front Cell Infect Microbiol.* 2012;2:150.

7. Sato H. Countermeasures and vaccination against terrorism using smallpox: pre-event and post-event smallpox vaccination and its contraindications. *Environ Health Prev Med*. 2011;16:281–289.

8. Pavlin JA. Epidemiology of bioterrorism. *Emerg Infect Dis*. 1999;5:528–530.

9. Cieslak TJ, Henretig FM. Medical consequences of biological warfare: the ten commandments of management. *Mil Med*. 2001;166:11–12.

10. US Army Medical Research Institute of Infectious Diseases (USAMRIID), Centers for Disease Control and Prevention (CDC), and Food and Drug Administration (FDA). Biological warfare and terrorism: the military and public health response [transcript]. Satellite television broadcast student handbook. September 21–23, 1999.

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

12. Fine A, Layton M. Lessons from the West Nile viral encephalitis outbreak in New York City, 1999: implications for bioterrorism preparedness. *Clin Infect Dis*. 2001;32:277–282.

13. Inglesby TV. Anthrax: a possible case history. *Emerg Infect Dis*. 1999;5:556–560.

14. Steele KE, Linn MJ, Schoepp RJ, et al. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet Pathol*. 2000;37:208–224.

15. Ludwig GV, Calle PP, Mangiafico JA, et al. An outbreak of West Nile virus in a New York City captive wildlife population. *Am J Trop Med Hyg*. 2002;67:67–75.

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

17. Editorial team: probable human anthrax death in Scotland. *Euro Surveill*. 2006;11:pii=3025. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3025. Accessed January 13, 2014.

18. Dalton R. Genetic sleuths rush to identify anthrax strains in mail attacks. *Nature*. 2001;413:657–658.

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

20. Gursky E, Inglesby TV, O'Toole T. Anthrax 2001: observations on the medical and public health response. *Biosecur Bioterror*. 2003;1:97–110.

21. US Army Soldier and Biological Chemical Command, Biological Warfare Improved Response Program. *Criminal and Epidemiological Investigation Report.* Washington, DC: National Disaster Preparedness Office, Department of Defense; 2000.

22. Carus WS. *Working Paper: Bioterrorism and Biocrimes. The Illicit Use of Biological Agents Since 1900. February 2001 Revision*. Washington, DC: Center for Counterproliferation Research, National Defense University; 2001.

23. Wheelis M. Biological sabotage in World War I. In: Geissler E, van Courtland Moon JE, eds. *Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945*. Vol 18. Stockholm International Peace Research Institute, Chemical & Biological Warfare Studies. Oxford, Great Britain: Oxford University Press; 1999: Chap 3.

24. Pelton T. New plots, fresh fears, old germs. *The Baltimore Sun*. October 28, 2001.

25. Witcover J. *Sabotage at Black Tom: Imperial Germany's Secret War in America, 1914–1917*. Chapel Hill, NC: Algonquin Books of Chapel Hill; 1989.

26. Koenig R. *The Fourth Horseman: One Man's Mission to Wage the Great War in America*. New York, NY: Public Affairs; 2006.

27. Watson SA. The changing biological warfare threat: anti-crop and anti-animal agents. *Ann New York Acad Sci*. 1999;894:159–163.

28. Deen WA. Trends in American agriculture: their implications for biological warfare against crop and animal resources. *Ann N Y Acad Sci*. 1999;894:164–167.

29. US Department of Agriculture. Animal and Plant Health Inspection Service. National Animal Health Laboratory Network Web site. http://www.aphis.usda.gov/animal_health/nahln/. Accessed January 14, 2014.

30. US Department of Agriculture. Animal and Plant Health Inspection Service. Centers for Epidemiology and Animal Health Web site. http://www.aphis.usda.gov/about_aphis/programs_offices/veterinary_services/ceah.shtml. Accessed January 14, 2014.

31. Centers for Disease Control and Prevention. *CDC Estimates of Foodborne Illness in the United States*. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed January 11, 2014.

32. Centers for Disease Control and Prevention. *What is Salmonellosis?* http://www.cdc.gov/salmonella/general/. Accessed January 11, 2014.

33. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis*. 1999;5:607–625.

34. Voetsch AC, Van Gilder TJ, Angulo FJ, et al. FoodNet estimate of the burden of illness caused by nontyphoidal Salmonella infections in the United States. *Clin Infect Dis*. 2004; 38(Suppl 3):S127–S134.

35. 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.

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

37. Olson KB. Aum Shinrikyo: once and future threat? *Emerg Infect Dis*. 1999;5:513–516.

38. Clinehens NA. *Aum Shinrikyo and Weapons of Mass Destruction: A Case Study*. Maxwell Air Force Base, AL: Air Command and Staff College, Air University; 2000.

39. Takahashi H, Keim P, Kaufmann AF, et al. *Bacillus anthracis* incident, Kameido, Tokyo, 1993. *Emerg Infect Dis*. 2004;10:117–120.

40. Danzig R, Sageman M, Leighton T, et al. *Aum Shinrikyo: Insights Into How Terrorists Develop Biological and Chemical Weapons*. Washington, DC: Center for a New American Security; 2011.

41. Keim P, Smith KL, Keys C, Takahashi H, Kurata T, Kaufmann A. Molecular investigation of the Aum Shinrikyo anthrax release in Kameido, Japan. *J Clin Microbiol*. 2001;39:4566–4567.

42. Kolavic SA, Kimura A, Simons SL, et al. An outbreak of Shigella dysenteriae type 2 among laboratory workers due to intentional food contamination. *JAMA*. 1997;278:396–398.

43. Reaves J. Anthrax: separating fear from fact. *Time.com*. October 12, 2001.

44. Centers for Disease Control and Prevention. Ongoing investigation of anthrax—Florida, October 2001. *MMWR Morb Mortal Wkly Rep*. 2001;50:877.

45. Chenault EA. Hunters should take precautions against anthrax. *AgNews*. College Station, TX: Texas A&M University System Agriculture Program; 2001.

46. Hsu VP, Lukacs SL, Handzel T, et al. Opening a Bacillus anthracis-containing envelope, Capitol Hill, Washington, DC: the public health response. *Emerg Infect Dis*. 2002;8:1039–1043.

47. Jernigan JA, Stephens DS, Ashford DA, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg Infect Dis*. 2001;7:933–944.

48. Griffith KS, Mead P, Armstrong GL, et al. Bioterrorism-related inhalational anthrax in an elderly woman—Connecticut, 2001. *Emerg Infect Dis*. 2003;9:681–688.

49. Centers for Disease Control and Prevention. Suspected cutaneous anthrax in a laboratory worker—Texas, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:279–281.

50. Butler JC, Cohen ML, Friedman CR, Scripp RM, Watz CG. Collaboration between public health and law enforcement: new paradigms and partnerships for bioterrorism planning and response. *Emerg Infect Dis*. 2002;8:1152–1156.

51. Tan CG, Sandhu HS, Crawford DC, et al. Surveillance for anthrax cases associated with contaminated letters, New Jersey, Delaware, and Pennsylvania, 2001. *Emerg Infect Dis*. 2002;8:1073–1077.

52. Williams AA, Parashar UD, Stoica A, et al. Bioterrorism-related anthrax surveillance—Connecticut, September–December, 2001. *Emerg Infect Dis*. 2002;8:1078–1082.

53. Heller MB, Bunning ML, France MEB, et al. Laboratory response to anthrax bioterrorism—New York City, 2001. *Emerg Infect Dis*. 2002;8:1096–1102.

54. Khan AS, Morse S, Lillibridge S. Public-health preparedness for biological terrorism in the USA. *Lancet*. 2000;356:1179–1182.

55. Hoffmaster AR, Fitzgerald CC, Ribot E, et al. Molecular subtyping of *Bacillus anthracis* and the 2001 bioterrorism-related anthrax outbreak, United States. *Emerg Infect Dis*. 2002;8:1111–1116.

56. Dewan PK, Fry AM, Laserson K, et al. Inhalational anthrax outbreak among postal workers—Washington, DC, 2001. *Emerg Infect Dis*. 2002;8:1066–1072.

57. Dull PM, Wilson KE, Kournikakis B, et al. Bacillus anthracis aerosolization associated with a contaminated mail sorting machine. *Emerg Infect Dis*. 2002;8:1044–1047.

58. Teshale EH, Painter J, Burr GA, et al. Environmental sampling for spores of *Bacillus anthracis*. *Emerg Infect Dis*. 2002;8:1083–1087.

59. Wein LM, Liu Y, Leighton TJ. HEPA/vaccine plan for indoor anthrax remediation. *Emerg Infect Dis*. 2005;11:69–76.

60. Jefferds MD, Laserson K, Fry AM, et al. Adherence to antimicrobial inhalational anthrax prophylaxis among postal workers—Washington, DC, 2001. *Emerg Infect Dis*. 2002;8:1138–1144.

61. Quinn SC, Thomas T, Kumar S. The anthrax vaccine and research: reactions from postal workers and public health professionals. *Biosecur Bioterror*. 2008;6:321–333.

62. US Department of Health and Human Services. *HHS Announces $1.1 Billion in Funding to States for Bioterrorism Preparedness*. Press release. Washington, DC: DHHS; 2002. http://archive.hhs.gov/news/press/2002pres/20020131b.html. Accessed January 14, 2013.

63. Conrad JL, Pearson JL. Improving epidemiology, surveillance, and laboratory capabilities. In: Levy BS, Sidel VW, eds. *Terrorism and Public Health*. New York, NY: Oxford University Press; 2003:270–285: Chap 14.

64. Bale JM, Bhattacharjee A, Croddy E, Pilch R. *Ricin Found in London: An al-Qa'ida Connection?* Monterey, CA: Monterey Institute of International Studies, Center for Nonproliferation Studies, Chemical and Biological Weapons Nonproliferation Program; 2003. http://cns.miis.edu/reports/ricin.htm. Accessed January 14, 2014.

65. Centers for Disease Control and Prevention. Investigation of a ricin-containing envelope at a postal facility—South Carolina, 2003. *MMWR Morb Mortal Wkly Rep*. 2003;52:1129–1131.

66. Frist: Ricin confirmed, but no illness reported. February 4, 2004. http://www.cnn.com/2004/US/02/03/senate.hazardous/. Accessed November 18, 2014.

67. Goldstein J. Woman from Texas is charged in ricin case. *New York Times*. June 7, 2013. http://www.nytimes.com/2013/06/08/nyregion/texas-woman-arrested-in-connection-with-ricin-laced-letters.html?_r=0. Accessed January 13, 2014.

68. US Army Medical Research Institute of Infectious Diseases. *Medical Management of Biological Casualties Handbook*. 7th ed. Fort Detrick, MD: USAMRIID; 2011.

69. Zelicoff A, Brillman J, Forslund DW, et al. The Rapid Syndrome Validation Project (RSVP). *Proc AMIA Symp*. 2001;771–775.

70. Alibek K, Handelman S. *Biohazard: The Chilling True Story of the Largest Biological Weapons Program in the World—Told from Inside by the Man Who Ran It*. New York, NY: Random House; 1999.

71. Woods CW, Ospanov K, Myrzabekov A, Favorov M, Plikaytis B, Ashford DA. Risk factors for human anthrax among contacts of anthrax-infected livestock in Kazakhstan. *Am J Trop Med Hyg*. 2004;71:48–52.

72. Brookmeyer R, Blades N, Hugh-Jones M, Henderson DA. The statistical analysis of truncated data: application to the Sverdlovsk anthrax outbreak. *Biostatistics*. 2001;2:233–247.

73. Guillemin J. *Anthrax: The Investigation of a Deadly Outbreak*. Berkeley, CA: University of California Press; 1999.

74. Abramova FA, Grinberg LM, Yampolskaya OV, Walker DH. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979. *Proc Natl Acad Sci U S A*. 1993;15:2291–2294.

75. Walker DH, Yampolska O, Grinberg LM. Death at Sverdlovsk: what have we learned? *Am J Pathol*. 1994;144:1135–1141.

76. Jackson PJ, Hugh-Jones ME, Adair DM, et al. PCR analysis of tissue samples from the 1979 Sverdlovsk anthrax victims: the presence of multiple *Bacillus anthracis* strains in different victims. *Proc Natl Acad Sci U S A*. 1998;95:1224–1229.

77. Letter of David Satcher to Donald W. Riegle, June 21, 1995. *Congressional Record*. V. 148, PT. 13, September 20, 2002. P. 17405.

78. Preston R. West Nile mystery, how did it get here. The CIA would like to know. *The New Yorker*. October 18-25, 1999:90–107.

79. Zilinskas RA. Iraq's biological weapons: the past as future? *JAMA*. 1997;278:418–424.

80. Centers for Disease Control and Prevention. Outbreak of West Nile-like viral encephalitis – New York, 1999. *MMWR Morb Mortal Wkly Rep*. 1999;48:845–849.

81. Enserink M. Groups race to sequence and identify New York virus. *Science.* 1999;286:206–207.

82. Mostashari F, Bunning ML, Kitsutani PT, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet*. 2001;358:261–264.

83. Hubalek Z, Halouzka J. West Nile fever—a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis*. 1999;5:643–650.

84. Savage HM, Ceianu C, Nicolescu G, et al. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. *Am J Trop Med Hyg*. 1999;61:600–611.

85. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science*. 1999;286:2333–2337.

86. Turell MJ, O'Guinn M, Oliver J. Potential for New York mosquitoes to transmit West Nile virus. *Am J Trop Med Hyg*. 2000;62:413–414.

87. Nasci RS, White DJ, Stirling H. West Nile virus isolates from mosquitoes in New York and New Jersey, 1999. *Emerg Infect Dis*. 2001;7:626–630.

88. Enserink M. New York's lethal virus came from Middle East, DNA suggests. *Science.* 1999;286:1450–1451.

89. Jia X-Y, Briese T, Jordan I, et al. Genetic analysis of West Nile New York 1999 encephalitis virus. *Lancet*. 1999;354:1971–1972.

90. Asnis DS, Conetta R, Teixeira AA, Waldman G, Sampson BA. The West Nile Virus outbreak of 1999 in New York: the Flushing hospital experience. *Clin Infect Dis*. 2000;30:413–418.

91. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. West Nile virus. *Lancet Infect Dis*. 2002;2:519–529.

92. Feldman KA, Stiles-Enos D, Julian K, et al. Tularemia on Martha's Vineyard: seroprevalence and occupational risk. *Emerg Infect Dis*. 2003;9:350–354.

93. Centers for Disease Control and Prevention. Tularemia—United States, 2001–2010. *MMWR Morb Mortal Wkly Rep*. 2013;62:963–966.

94. Teutsch SM, Martone WJ, Brink EW, et al. Pneumonic tularemia on Martha's Vineyard. *N Engl J Med*. 1979;301:826–828.

95. Greer WER. Pulmonary tularemia in Massachusetts: report of a case due to tick bites. *N Engl J Med*. 1948;238:355–356.

96. Yeatter RE, Thompson DH. Tularemia, weather and rabbit populations. *Bull Illinois Natural History Survey*. 1952;25:351–382.

97. Dembek ZF, Buckman RL, Fowler SK, Hadler JL. Missed sentinel case of naturally occurring pneumonic tularemia outbreak: lessons for detection of bioterrorism. *J Am Board Fam Pract*. 2003;16:339–342.

98. Fraser C, Riley S, Anderson RM, Ferguson NM. Factors that make an infectious disease controllable. *Proc Natl Acad Sci U S A*. 2004;101:6146–6151.

99. Grunow R, Finke EJ. A procedure for differentiating between the intentional release of biological warfare agents and natural outbreaks of disease: its use in analyzing the tularemia outbreak in Kosovo in 1999 and 2000. *Clin Microbiol Infect*. 2002;8:510–521.

100. World Health Organization. Outbreak news. *Weekly Epidemiol Record*. 2000;75:133.

101. Prinzing F. War pestilences. In: Westergaard H, ed. *Epidemics Resulting From Wars*. Oxford, Great Britain: Clarendon Press; 1916: Chap 1.

102. Prinzing F. The time before the thirty years' war. In: Westergaard H, ed. *Epidemics Resulting From Wars*. Oxford, Great Britain: Clarendon Press; 1916: Chap 2.

103. Rotz LD, Khan AS, Lillibridge SR, Ostroff SM, Hughes JM. Public health assessment of potential biological terrorism agents. *Emerg Infect Dis*. 2002;8:225–230.

104. Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA*. 2001;285:2763–2773.

105. Gurycova D. First isolation of *Francisella tularensis* subsp. *tularensis* in Europe. *Eur J Epidemiol*. 1998;14:797–802.

106. Reintjes R, Dedushal I, Gjini A, et al. Tularemia outbreak investigation in Kosovo: case control and environmental studies. *Emerg Infect Dis*. 2002;8:69–73.

107. Tarnvik A, Priebe HS, Grunow R. Tularemia in Europe: an epidemiological overview. *Scand J Infect Dis*. 2004;36:350–355.

108. Croddy E, Krcalova S. Tularemia, biological warfare, and the battle for Stalingrad (1942–1943). *Mil Med*. 2001;166:837–838.

109. Angelakis E, Raoult D. Q fever. *Vet Microbiol*. 2010;140:297–309.

110. Faix DJ, Harrison DJ, Riddle MS, et al. Outbreak of Q fever among US military in western Iraq, June–July 2005. *Clin Infect Dis*. 2008;46:e65–e68.

111. Anderson AD, Smoak B, Shuping E, Ockenhouse C, Petruccelli B. Q fever and the US military. *Emerg Infect Dis*. 2005;11:1320–1322.

112. Leung-Shea C, Danaher PJ. Q fever in members of the United Stated armed forces returning from Iraq. *Clin Infect Dis*. 2006;43:e77–e82.

113. Gleeson TD, Decker CF, Johnson MD, Harztell JD, Mascola JR. Q fever in US military returning from Iraq. *Am J Med*. 2007;120:e11–e12.

114. Ferrante MA, Dolan MJ. Q fever meningoencephalitis in a soldier returning from the Persian Gulf War. *Clin Infect Dis*. 1993;16:489–496.

115. Anderson AD, Baker TR, Littrell AC, Mott RL, Niebuhr DW, Smoak BL. Seroepidemiologic survey for *Coxiella burnetii* among hospitalized US troops deployed to Iraq. *Zoonoses Public Health*. 2011;58:276–283.

116. Royal J, Riddle MS, Mohareb E, Monteville MR, Porter CK, Faix DJ. Seroepidemiologic survey for *Coxiella burnetii* among US military personnel deployed to Southwest and Central Asia in 2005. *Am J Trop Med Hyg*. 2013;89:991–995.

117. Bailey MS, Trinick TR, Dunbar JA, et al. Undifferentiated febrile illnesses amongst British troops in Helmand, Afghanistan. *J R Army Med Corps*. 2011;157:150–155.

118. Havas KA, Burkman K. A comparison of the serological evidence of *Coxiella burnetii* exposure between military working dogs and feral canines in Iraq. *Mil Med*. 2011;176:1101–1103.

119. Hartzell JD, Gleeson T, Scoville S, Massung RF, Wortmann G, Martin GJ. Practice guidelines for the diagnosis and management of patients with Q fever by the Armed Forces Infectious Diseases Society. *Mil Med*. 2012;177:484–494.

120. US Department of Homeland Security. *Health Threats Resilience Division*. https://www.dhs.gov/health-threats-resilience-division. Accessed January 14, 2014.

121. Garza A. *The Truth About BioWatch: The Importance of Early Detection of a Potential Biological Attack*. Washington, DC: US Department of Homeland Security; July 12, 2012. http://www.dhs.gov/blog/2012/07/12/truth-about-biowatch. Accessed January 14, 2014.

122. Sosin DM. Draft framework for evaluating syndromic surveillance systems. *J Urban Health*. 2003;80(2 Suppl 1):i8–i13.

123. Henning KJ. What is syndromic surveillance? *MMWR Morb Mortal Wkly Rep*. 2004;53(Suppl):5–11.

124. Sosin DM. Syndromic surveillance: the case for skillful investment. *Biosecur Bioterror*. 2003;1:247–253.

125. American Public Health Association, Skoll Global Threats Fund, Boston Children's Hospital, Health Map. *Flu near you do you have it in you?* https://flunearyou.org/. Accessed January 14, 2014.

126. Das D, Weiss D, Mostashari F, et al. Enhanced drop-in syndromic surveillance in New York City following September 11, 2001. *J Urban Health*. 2003;80(Suppl 1):i76–i88.

127. Schumacher M, Nohre L, Santana S. Partial evaluation of a drop-in bioterrorism surveillance system in Phoenix, Arizona. *J Urban Health*. 2003;80(Suppl 1):i118.

128. UK Health Protection Agency. *Syndromic Surveillance Report. London 2012 Olympic and Paralympic Games*. http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317137703688. Accessed January 14, 2014.

129. Goss L, Carrico R, Hall C, Humbaugh K. A day at the races: communitywide syndromic surveillance during the 2002 Kentucky Derby festival. *J Urban Health*. 2003;80(Suppl 1):i124.

130. Suzuki S, Ohyama T, Taniguchi K, et al. Web-based Japanese syndromic surveillance for FIFA World Cup 2002. *J Urban Health*. 2003;80(Suppl 1):i123.

131. Burkom H. Biosurveillance applying scan statistics with multiple, disparate data sources. *J Urban Health*. 2003;80(Suppl1):i57–i65.

132. *North Carolina Disease Event Tracking and Epidemiologic Collection Tool*. Chapel Hill, NC: NCDetect. http://www.ncdetect.org/. Accessed January 20, 2014.

133. *Real-time Outbreak and Disease Surveillance Laboratory*. Pittsburgh, PA: University of Pittsburgh. https://www.rods.pitt.edu/site/content/category/8/22/36/. Accessed January 20, 2014.

134. *EpiCenter*. Pittsburgh, PA: Health Monitoring Systems. http://www.hmsinc.com/service/epicenter.html. Accessed January 20, 2014.

135. *Biosense 2.0*. Atlanta, GA: Centers for Disease Control and Prevention. http://www.cdc.gov/biosense/biosense20.html. Accessed January 14, 2014.

136. Reuter S, Ellington MJ, Cartwright EJ, et al. Rapid bacterial whole-genome sequencing to enhance diagnostic and public health microbiology. *JAMA Intern Med*. 2013;173:1397–1404.

137. Zhang Y, Lopez-Gatell H, Alpuche-Aranda CM, Stoto MA. Did advances in global surveillance and notification systems make a difference in the 2009 H1N1 pandemic?–a retrospective analysis. *PLoS One*. 2013;8:e59893.

138. Meldrum C, Doyle MA, Tothill RW. Next-generation sequencing for cancer diagnostics: a practical perspective. *Clin Biochem Rev*. 2011;32:177–195.

139. Roetzer A, Diel R, Kohl TA, et al. Whole genome sequencing versus traditional genotyping for investigation of a Mycobacterium tuberculosis outbreak: a longitudinal molecular epidemiological study. *PLoS Med*. 2013;10(2):e1001387.

140. Klee SR, Tyczka J, Ellerbrok H, et al. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. *BMC Microbiol*. 2006;6:2.

141. Willems H, Thiele D, Frolich-Ritter R, Krauss H. Detection of *Coxiella burnetii* in cow's milk using the polymerase chain reaction (PCR). *Zentralbl Veterinarmed B*. 1994;41:580–587.

142. Zhou J, Chu H, Li C, et al. Active replication of Middle East respiratory syndrome coronavirus replication and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis. *J Infect Dis*. 2014;209:1331–1342.

143. Chan K, Marras SA, Parveen N. Sensitive multiplex PCR assay to differentiate Lyme spirochetes and emerging pathogens *Anaplasma phagocytophilum* and *Babesia microti*. *BMC Microbiol*. 2013;13:295.

144. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Sci*. 2000;288:1432–1435.

145. Memish ZA, Mishra N, Olival KJ, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. *Emerg Infect Dis*. 2013;19:1819–1823.

146. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science.* 1988;239:487–491.

147. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 1977;74:5463–5467.

148. Lakey DL, Melville SK, Robbins AS. *Emerging Technologies and Advancements in HIV and AIDS Surveillance and Testing to Enhance Surveillance, Prevention and Treatment of HIV and AIDS Infection*. Austin, TX: Texas Department of State Health Services; January 1, 2009.

149. Lemon SM, Hamburg MA, Sparling PF, Choffnes ER, Mack A. *Global Infectious Disease Surveillance and Detection: Assessing the Challenges - Finding Solutions, Workshop Summary*. Washington, DC: National Academies Press; 2007.

150. Sabat AJ, Budimir A, Nashev D, et al. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Euro Surveill*. 2013;18:20380.

151. DeLisi C. Overview of human genome research. *Basic Life Sci*. 1988;46:5–10.

152. DeLisi C. Meetings that changed the world: Santa Fe 1986: human genome baby-steps. *Nature*. 2008;455:876–877.

153. Koser CU, Ellington MJ, Cartwright EJ, et al. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. *PLoS Pathog*. 2012;8:e1002824.

154. Mardis ER. A decade's perspective on DNA sequencing technology. *Nature*. 2011;470:198–203.

155. Watterstrand K. *DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP).* Bethesda, MD: National Institutes of Health; 2013.

156. Zhou X, Ren L, Li Y, Zhang M, Yu Y, Yu J. The next-generation sequencing technology: a technology review and future perspective. *Sci China Life Sci*. 2010;53:44–57.

157. Garten RJ, Todd Davis C, Russell CA, et al. Antigenic and genetic characteristics of the early isolates of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science*. 2009;352:197–201.

158. Liu D, Shi W, Shi Y, et al. Origin and diversity of novel avian influenza A H7N9 viruses causing human infection: phylogenetic, structural, and coalescent analyses. *Lancet*. 2013;381:1926–1932.

159. Bielaszewska M, Mellmann A, Zhang W, et al. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect Dis*. 2011;11:671–676.

160. Rohde H, Qin J, Cui Y, et al. Open-source genomic analysis of Shiga-toxin-producing E. coli O104:H4. *N Engl J Med*. 2011;365:718–724.

161. Staff B-IN. *German Teams, BGI and Life Technologies Identify Deadly European E. coli Strain*. Needham, MA: Cambridge Healthtech Institute; 2011.

162. Dormitzer PR, Suphaphiphat P, Gibson DG, et al. Synthetic generation of influenza vaccine viruses for rapid response to pandemics. *Sci Transl Med*. 2013;5:185ra68.

163. Anthony SJ, Epstein JH, Murray KA, et al. A strategy to estimate unknown viral diversity in mammals. *MBio*. 2013;4:e00598-e00513.

164. Morse SS, Mazet JAK, Woolhouse M, et al. Prediction and prevention of the next pandemic zoonosis. *Lancet*. 2012;380:1956–1965.

165. Bengis RG, Leighton FA, Fischer JR, Artois M, Morner T, Tate CM. The role of wildlife in emerging and re-emerging zoonoses. *Rev Sci Tech*. 2004;23:497–511.

166. Mackenzie JS, Jeggo M. Reservoirs and vectors of emerging viruses. *Curr Opin Virol*. 2013;3:170–179.

167. EcoHealth Alliance. *USAID's PREDICT Program Releases a New Forecasting Framework for Disease Emergence*. http://www.ecohealthalliance.org/news/179-usaids_predict_program_releases_a_new_forecasting_framework_for_disease_emergence. Accessed December 30, 2013.

168. Fouchier RA, Garcia-Sastre A, Kawaoka Y. Pause on avian flu transmission studies. *Nature*. 2012;481:443.

169. Fouchier RA, Herfst S, Osterhaus AD. Public health and biosecurity. Restricted data on influenza H5N1 virus transmission. *Science*. 2012;335:662–663.

170. Malakoff D. Avian influenza. Critics skeptical as flu scientists argue for controversial H7N9 studies. *Science*. 2013;341:601.

171. Fouchier RA, Kawaoka Y, Cardona C, et al. Gain-of-function experiments on H7N9. *Sci*. August 7, 2013.

172. Imai M, Watanabe T, Hatta M, et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature*. 2012;486:420–428.

173. Imai M, Herfst S, Sorrell EM, et al. Transmission of influenza A/H5N1 viruses in mammals. *Virus Res*. 2013;178:15–20.

174. Richard M, Schrauwen EJ, de Graaf M, et al. Limited airborne transmission of H7N9 influenza A virus between ferrets. *Nature*. 2013;501:560–563.

175. Buchholz U, Bernard H, Werber D, et al. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *N Engl J Med*. 2011;365:1763–1770.

176. Tremlett G, Pidd H. Germany admits Spanish cucumbers are not to blame for E coli outbreak. May 31, 2011. The Guardian. http://www.theguardian.com/uk/2011/may/31/e-coli-deaths-16-germany-sweden. Accessed November 18, 2014.

177. E. coli cucumber scare: Spain angry at German claims. *BBC News Europe*. May 31, 2011. http://www.bbc.co.uk/news/world-europe-13605910. Accessed January 10, 2014.

178. Sandman PM, Lanard J. Explaining and proclaiming uncertainty: risk communication lessons from Germany's deadly E. coli outbreak. The Peter Sandman Risk Communication Website. August 14, 2011. http://www.psandman.com/col/GermanEcoli.htm. Accessed November 18, 2014.

179. Qin J, Cui Y, Zhao X, et al. Identification of the Shiga toxin-producing Escherichia coli O104:H4 strain responsible for a food poisoning outbreak in Germany by PCR. *J Clin Microbiol*. 2011;49:3439–3440.

180. Rasko DA, Webster DR, Sahl JW, et al. Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med*. 2011;365:709–717.

181. King LA, Nogareda F, Weill FX, et al. Outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 associated with organic fenugreek sprouts, France, June 2011. *Clin Infect Dis*. 2012;54:1588–1594.

182. Jacob D, Sauer U, Housley R, et al. Rapid and high-throughput detection of highly pathogenic bacteria by Ibis PLEX-ID technology. *PLoS One*. 2012;7:e39928.

183. Federation of American Scientists. *Introduction to Biological Weapons*. Washington, DC: Federation of American Scientists; 2007.

184. Committee on Scientific Milestones for the Development of a Gene-Sequence-Based Classification System for the Oversight of Select Agents; National Research Council. *Sequence-Based Classification of Select Agents: A Brighter Line*. Washington, DC: The National Academies Press; 2010.

185. National Institutes of Health. Notice Pertinent to the March 2013 Revisions of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid (NIH Guidelines). http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html. Accessed November 18, 2014.

186. National Science Advisory Board for Biosecurity. *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents.* Bethesda, MD: NSABB; 2006.

187. Centers for Disease Control and Prevention. *Imported Plague New York City, 2002.* Atlanta, GA: CDC; 2003.

188. National Center for Emerging and Zoonotic Infectious Diseases. Centers for Disease Control and Prevention. Florida retiree gets—and survives—anthrax: a medical victory remains a medical mystery. *Anthrax Resources*. Atlanta, GA: CDC; October 24, 2013.

189. Ortiz E. 7-year-old Colorado girl infected with bubonic plague leaves hospital. *New York Daily News*. September 12, 2012. http://www.nydailynews.com/life-style/health/7-year-old-colorado-girl-infected-bubonic-plague-leave-hospital-article-1. 1155802. Accessed November 18, 2014.

190. Dockterman E. The black death returns: Oregon man in critical condition with the plague. *Time Inc*. June 5, 2012.

191. McNeil DG. United States ranks 11th in plague cases worldwide. *New York Times*. September 16, 2013.

192. Dubois S. Oregon plague: woman contracted disease from cat. *Huffington Post*. September 15, 2012. http://www.huffingtonpost.com/2012/09/15/oregon-plague-woman-disease-cat_n_1887053.html. Accessed October 3, 2014.

193. Associated Press. Officials: tests show presence of anthrax in second Florida man and at newspaper building. *The Daily Star*. October 8, 2001.