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Epidemiologic Investigation for Public Health, Biodefense, and Forensic Microbiology

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Microorganisms are very efficient at infecting humans, using a number of different strategies and mechanisms. The deliberate dissemination of a biological agent by many of these same mechanisms presents the latest challenge to public health. The deliberate dissemination will often be obvious; however, identifying the covert dissemination of a biological may present challenges. Nonetheless, a thorough investigation integrating epidemiologic data and molecular typing will help to differentiate between a naturally occurring disease outbreak and one resulting from an act of terrorism.

INTRODUCTION

Epidemiology is the study of how disease is distributed in populations and of the factors that influence this distribution.¹ More broadly, it is the study of the distribution and determinants of health-related states or events in specified populations and the application of this study to control health problems.² Epidemiology is based on the premise that disease, illness, and ill health are not randomly distributed in a population and that individuals have certain characteristics (e.g., genetic or environmental) that predispose to, or protect against, a variety of different diseases. The specific objectives of epidemiology¹ are to: (1) identify the etiology or cause of a disease and the factors that

increase a person's risk for a disease, (2) determine the extent of disease found in the community, (3) study the natural history and prognosis of disease, (4) evaluate new preventive and therapeutic measures and new modes of health care delivery, and (5) provide a foundation for developing public policy and regulations. This chapter will discuss how epidemiology can be used to identify the source of diseases caused by microorganisms or toxins.

DYNAMICS OF DISEASE TRANSMISSION

Disease has been classically described as the result of an epidemiological triad, where disease results from the interaction of the human host, an infectious agent or toxin, and the environment that promotes the exposure.¹ In some instances, an arthropod vector such as a mosquito or tick is involved. Among the assumptions necessary for this interaction to take place is that there is a susceptible host. The susceptibility of the host is influenced by a variety of factors including genetic, nutritional, and immunological factors. The bacteria, viruses, fungi, and parasites responsible for disease can be transmitted in either a direct or indirect fashion (Table 8.1). Different organisms spread in different ways, and the potential of a given organism to spread and produce outbreaks depends on the characteristics of the organism and the route by which it is transmitted from person to person.

Diseases can be defined as endemic, epidemic, and pandemic. **Endemic** can be defined as either the habitual presence of a disease within a given geographical area, or as the usual occurrence of a given disease within such an area. **Epidemic** can be defined as the occurrence in a community or region of disease, clearly in excess of normal expectancy, and derived from a common source or from a propagated source. **Pandemic** refers to a worldwide epidemic. The usual or expected level of a disease is determined through ongoing **surveillance**.

Microorganisms are very efficient at infecting humans, using a number of different strategies and mechanisms. These are exemplified both by the various

TABLE 8.1 Modes of agent transmission (modified from ref. 1)

Horizontal

Direct transmission

Contact (person-to-person)

Indirect transmission

Common vehicle

Single exposure

Multiple exposures

Continuous exposure

Vertical (transmission from one generation to another)

strategies devised by the microbe to survive prior to infecting a host such as sporulation or harboring in drought-resistant mosquito eggs, and by the various modes of transmission, e.g., direct contact (including large droplets) or indirect contact with fomites, or by insect vectors, and airborne via small particle droplets.³ Natural experiments, however, have highlighted the true diversity in the abilities of microorganisms to infect humans and animals: *Salmonella* outbreaks due to contaminated alfalfa sprouts⁴ and to ice cream made from milk that was contaminated in a tanker that had previously contained raw eggs,⁵ legionellosis associated with grocery misters,⁶ Severe Acute Respiratory Syndrome (SARS) and Ebola hemorrhagic fever in healthcare facilities,^{7,8} the translocation of Rift Valley fever virus from Africa to the Arabian Peninsula and West Nile virus to the U.S.,^{9,10} and pneumonic tularemia on Martha's Vineyard from mowing over a rabbit.¹¹ These few examples are a semblance of the seemingly endless list of novel ways that agents and their vectors are spread. The ability to exploit newly created biological conditions is the hallmark and challenge of emerging infections.¹²

OUTBREAK INVESTIGATION

The occurrence of a disease at more than an endemic level may stimulate an investigation during which investigators may ask three questions. **Who** was attacked by the disease? The answers to this question will help to identify those characteristics of the human host that are closely related to disease risk. **When** did the disease occur? Some diseases occur with a certain periodicity. This question is also addressed by examining trends of disease incidence over time. **Where** did the cases rise? The answers to the previous questions lead to determining the **how** and **why** of an outbreak. Disease is not randomly distributed in time and place. These questions are central to virtually all outbreak investigations. The investigation of an outbreak may be primarily deductive (i.e., reasoning from premises or propositions proved antecedently) or inductive (i.e., reasoning from particular facts to a general conclusion), or it may be a combination of both. Important considerations in the investigation of acute outbreaks of infectious disease include: (1) determining that an outbreak has in fact occurred, (2) defining the population at risk, (3) determining the method of spread and reservoir, and (4) characterizing the agent. The steps commonly used for investigating an outbreak are shown in Table 8.2.

DELIBERATE INTRODUCTION OF A BIOLOGICAL AGENT

Deliberate dissemination of a biological agent via a number of different routes, including air, water, food, and infected vectors presents the latest challenge to

TABLE 8.2 Commonly used steps in the investigation of an infectious disease outbreak (modified from ref. 1)

Step 1. Define the epidemic.

Define the “numerator” (i.e., cases):

- Clinical features: is the disease known?
- What are its serologic or cultural aspects?
- Are the causes partially understood?

Define the “denominator”:

- What is the population at risk of developing disease?
- Calculate the attack rates.

Step 2. Examine the distribution of cases by the following:

Time: Look for time-place interactions.

Place

Person: Examine the risk in subgroups of the affected population according to personal characteristics: sex, age, residence, occupation, social group, etc.

Step 3. Look for combinations (interactions) of relevant variables.

Step 4. Develop hypotheses based on the following:

Existing knowledge (if any) of the disease

Analogy to diseases of known etiology

Step 5. Test hypotheses.

Further analyze existing data (case-control studies)

Collect additional data

Step 6. Recommend control measures.

Control of present outbreak

Prevention of future similar outbreaks

the global public health. The deliberate nature of such dissemination will often be obvious, as in the case of multiple mailed letters containing highly refined anthrax spores. However, some forms of bioterrorism may be more covert. For example, the deliberate contamination of salad bars in The Dalles, Oregon in 1984 by a religious cult in an effort to test their ability to incapacitate the local population prior to an election sickened more than 750 persons.¹³ The outbreak was specifically excluded as bioterrorism during the initial investigation, and only recognized as such following a tip from an informant.¹⁴ Given the natural ability of infectious agents to emerge, the Oregon outbreak serves to highlight the difficulties in determining a characteristic signature for an infectious disease outbreak resulting from deliberate transmission.

The difficulties in identifying a covert dissemination of a biological agent are exemplified by the investigation in The Dalles of a foodborne outbreak with a very unusual pattern and a rare strain of *Salmonella typhimurium*. Although the possibility of intentional contamination was considered early in the investigation, it was specifically excluded for the following reasons: (1)

such an event had never been reported previously, (2) no one claimed responsibility, (3) no disgruntled employee was identified, (4) no motive was apparent, (5) the epidemic curve suggested multiple exposures, which was presumed to be unlikely behavior for a saboteur, (6) law enforcement officials failed to establish a recognizable pattern of unusual behavior, (7) a few employees had onset of illness before the patrons, suggesting a possible source of infection, (8) the outbreak was biologically plausible—even if highly unlikely, and, (9) it is not unusual to not be able to find a source in even highly investigated outbreaks. Although one of the initial reasons to exclude terrorism (i.e., no prior incidents) is no longer applicable, based on similar actions since 1984, determining if an unusual outbreak is biologically plausible will remain a challenge. In this context, it is important to remember that the first case of inhalation anthrax identified in Florida in 2001 was initially thought to be natural. It is clear from the two documented cases of bioterrorism in the U.S.—the 1984 Oregon salmonella outbreak and the 2001 anthrax attack—that a terrorist will not necessarily announce his/her intentions or take credit for such an attack.^{13,15} Similarly, divining the motives behind an attack should be abandoned as a public health tool to assess whether an outbreak is natural or deliberate in nature. Fortunately, there are a number of epidemiologic clues that in themselves or in combination may suggest that an outbreak is deliberate. It is essential to make this determination not only from the law enforcement standpoint to prevent future such actions, but to protect the public health. There is a very short “window of opportunity” in which to implement postexposure prophylaxis for many of the agents likely to be used for bioterrorism.¹⁶ Even when postexposure prophylaxis may be unavailable or of limited utility, ascertaining the deliberate nature of an attack can allow for more effective postexposure planning for potential casualties and to improve surveillance for additional events. Therefore, it is critical that all outbreaks be rapidly investigated and assessed for whether they are of deliberate origin.

A set of epidemiologic clues (Table 8.3) has been proposed by the Department of Health and Human Service’s Centers for Disease Control and Prevention (CDC) in collaboration with the Federal Bureau of Investigation (FBI).¹⁷ These clues are based on distinctive epidemiology and laboratory criteria of varying specificity to evaluate whether an outbreak may be of deliberate origin. The clues focus on aberrations in the typical characterization of an outbreak by person, place, and time in addition to consideration of the microorganism. Some of the clues, such as a community-acquired case of smallpox, are quite specific for bioterrorism whereas others, such as similar genetic typing of an organism, may simply denote a natural outbreak. A combination of clues, especially those that suggest suspicious point source outbreaks, will increase the probability that the event is likely due to bioterrorism. Although these clues

TABLE 8.3 Epidemiologic clues that may signal a biologic or chemical terrorist attack (modified from ref. 17)

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1. Single case of disease caused by an uncommon agent (e.g., glanders, smallpox, viral hemorrhagic fever, inhalation or cutaneous anthrax) without adequate epidemiologic explanation
 2. Unusual, atypical, genetically engineered, or antiquated strain of an agent (or antibiotic-resistance pattern)
 3. Higher morbidity and mortality in association with a common disease or syndrome or failure of such patients to respond to usual therapy
 4. Unusual disease presentation (e.g., inhalation anthrax or pneumonic plague)
 5. Disease with an unusual geographic or seasonal distribution (e.g., plague in a nonendemic area, influenza in the summer)
 6. Stable endemic disease with an unexplained increase in incidence (e.g., tularemia, plague)
 7. Atypical disease transmission through aerosols, food, or water, in a mode suggesting sabotage (i.e., no other possible physical explanation)
 8. No illness in persons who are not exposed to common ventilation systems (have separate closed ventilation systems) when illness is seen in persons in close proximity who have a common ventilation system
 9. Several unusual or unexplained diseases coexisting in the same patient without any other explanation
 10. Unusual illness that affects a large, disparate population (e.g., respiratory disease in a large heterogeneous population may suggest exposure to an inhaled pathogen or chemical agent)
 11. Illness that is unusual (or atypical) for a given population or age group (e.g., outbreak of measles-like rash in adults)
 12. Unusual pattern of death or illness among animals (which may be unexplained or attributed to an agent of bioterrorism) that precedes or accompanies illness or death in humans
 13. Unusual pattern of death or illness in humans that precedes or accompanies illness or death in animals (which may be unexplained or attributed to an agent of bioterrorism)
 14. Ill persons who seek treatment at about the same time (point source with compressed epidemic curve)
 15. Similar genetic type among agents isolated from temporally or spatially distinct sources
 16. Simultaneous clusters of similar illness in noncontiguous areas, domestic or foreign
 17. Large numbers of cases of unexplained diseases or deaths
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are an important set of criteria to help evaluate outbreaks, no list will replace sound epidemiology to assess an outbreak.

It is important to note that epidemiologic clues can only be assessed in the context of a rapid and thorough epidemiologic investigation. Not surprisingly, surveillance to identify increases in disease incidence is both the first step and the cornerstone of bioterrorism epidemiology. The majority of the clues described in Table 8.3 simply suggest an unusual cluster of cases. They have been reorganized by specificity to trigger increasingly broader investigations

TABLE 8.4 Recommendations for level of public health involvement for investigation of potential biologic or chemical terrorism (modified from ref. 17)

Initial investigation at the local level

- a. Higher morbidity and mortality than expected, associated with a common disease or syndrome
- b. Disease with an unusual geographic or seasonal distribution
- c. Multiple unusual or unexplained disease entities coexisting in the same patient
- d. Unusual illness in a population (e.g., renal disease in a large population which may be suggestive of toxic exposure to an agent such as mercury)
- e. Ill persons seeking treatment at about the same time
- f. Illness in persons suggesting a common exposure (e.g., same office building, meal, sporting event, or social event)

Continued investigation with involvement of the state health department and/or Centers for Disease Control and Prevention

- a. At least a single, definitively diagnosed case(s) with one of the following:
 - Uncommon agent or disease
 - Illness due to a genetically altered organism
 - b. Unusual, atypical, or antiquated strain of agent
 - c. Disease with unusual geographic, seasonal, or “typical patient” distribution
 - d. Endemic disease with unexplained increase in incidence
 - e. No illness in persons not exposed to common ventilation systems
 - f. Simultaneous clusters of similar illness in non-contiguous areas, domestic or foreign
 - g. Cluster of patients with similar genetic type among agents isolated from temporally or spatially distinct sources
-

by state and federal public health officials and to alert law enforcement authorities (Tables 8.4 and 8.5). However, even the most specific of clues may signal a new natural outbreak. For example, the recent community outbreak of individuals with smallpox-like lesions in the Midwest may, on first blush, have indicated the deliberate release of smallpox virus. However, a thorough integrated epidemiologic and laboratory investigation identified the disease as monkeypox, an exotic disease in the U.S., which in itself should suggest bioterrorism.¹⁸ Affected individuals were infected by prairie dogs purchased as pets, which had acquired their infection while co-housed with infected Giant Gambian rats that had recently been imported from Ghana, and not from deliberate dissemination. Similarly, other emerging infectious diseases such as West Nile encephalitis and SARS would appropriately meet the criteria for suspect bioterrorism and require a thorough investigation.

MOLECULAR STRAIN TYPING

The microbiology laboratory has made significant contributions to the epidemiology of infectious diseases. The repeated isolation of a specific

TABLE 8.5 Considerations for notifying law enforcement of a possible biologic or chemical terrorism initial investigation at the local level (modified from ref. 17)

Immediate notification of the FBI when:

- a. Notification is received from individual or group that a terrorist attack has occurred or will occur.
- b. A potential dispersal/delivery device such as munition or sprayer or questionable material is found.

Notification of the FBI as soon as possible after an investigation confirms the following:

- a. Illness due to unexplained aerosol, food, or water transmission.
- b. At least a single, definitively diagnosed case(s) with one of the following:
 - Uncommon agent or disease occurring in a person with no other explanation
 - Illness due to a genetically altered organism

Notification of the FBI after an investigation confirms the following (with no plausible natural explanation):

- a. Disease with an unusual geographic, seasonal, or “typical patient” distribution
 - b. Unusual, atypical, or antiquated strain of agent
 - c. Simultaneous clusters of similar illness in noncontiguous areas, domestic or foreign
 - d. Clusters of patients presenting with similar genetic type among agents isolated from temporally or spatially distinct sources
-

microorganism from patients with a given disease or syndrome has helped to prove infectious etiologies. In addition, the isolation and identification of microorganisms from animals, vectors, and environmental sources has been invaluable in identifying reservoirs and verifying modes of transmission. In dealing with an infection, it is often necessary to identify the species of the infecting microorganism in order to prescribe effective therapy. Many of the techniques that have evolved for such purposes are both rapid and accurate but, in general, do not provide the kind of genetic discrimination necessary for addressing epidemiologic questions. The epidemiology of many infectious diseases is becoming more complex. Fortunately, typing methods for bacteria, fungi, protozoa, and viruses have evolved to meet this challenge. Historically, the typing methods that have been used in epidemiologic investigations fall into two broad categories: *phenotypic* methods and *genotypic* methods. Phenotypic methods are those methods that characterize the products of gene expression in order to differentiate strains. For example, the use of biochemical profiles to discriminate between genera and species of bacteria is used as a diagnostic method, but can also be used for biotyping. Other methods, such as phage typing, can be used to discriminate among groups within a bacterial species. Biotyping emerged as a useful tool for epidemiologic investigations in the 1960s and early 1970s, while phage typing of bacteria and serological typing of bacteria and viruses has been used for decades. Today, the majority of these tests are considered inadequate for epidemiologic purposes. First, they

TABLE 8.6 Characteristics of phenotypic typing methods (modified from ref. 23)

Typing System	Proportion of Strains Typeable	Reproducibility	Discriminatory Power	Ease of Interpretation	Ease of Performance
Biotyping	All	Poor	Poor	Moderate	Easy
Antimicrobial susceptibility patterns	All	Good	Poor	Easy	Easy
Serotyping	Most	Good	Fair	Moderate	Moderate
Bacteriophage or pyocin typing	Some	Good	Fair	Difficult	Difficult
MLEE ¹	All	Excellent	Excellent	Moderate	Moderate

¹MLEE, multilocus enzyme electrophoresis.

do not provide enough unrelated parameters to obtain a good reflection of genotype. For example, serotyping of *Streptococcus pneumoniae* discriminates among only a limited number of groups. In addition, some virus species, such as human cytomegalovirus and measles virus, cannot be divided into different types or subtypes by serology, because significant antigenic differences do not exist. Second, the expression of many genes is affected by spontaneous mutations, environmental conditions, and by developmental programs or reversible phenotypic changes, such as high-frequency phenotypic switching. Because of this, many of the properties measured by phenotypic methods have a tendency to vary, and for the most part they have been replaced by genotypic methods. The one major exception is multilocus enzyme electrophoresis (MLEE),^{19,20} which is a robust phenotypic method that performs comparably with many of the most effective DNA-based methods.^{21,22} Characteristics of selected phenotypic methods are presented in Table 8.6. These methods have been characterized by: *typeability*, which is the ability of the technique to assign an unambiguous result (i.e., type) to each isolate; *reproducibility*, which is when a method yields the same results upon repeat testing of a bacterial strain; *discriminatory power*, which is the ability of the method to differentiate among epidemiologically unrelated isolates; *ease of interpretation*, which refers to the effort and experience required to obtain useful, reliable typing information using a particular method; and *ease of performance*, which reflects the cost of specialized reagents and equipment, technical complexity of the method, and the effort required to learn and implement the method.

Extremely sensitive and specific molecular techniques have recently been developed to facilitate epidemiologic studies. Our ability to use these

TABLE 8.7 Molecular characterization of genetic diversity at different hierarchical levels (modified from ref. 24)

Function	Purpose	Regions of DNA
Discrimination above level of species	Taxonomy/evolution	Highly conserved coding regions (e.g., rDNA)
Discrimination between species	Taxonomy/diagnosis/epidemiology	Moderately conserved regions
Discrimination between intraspecific variants/strains	Population genetics	Variable regions
Discrimination between individual isolates/clonal lineages	“Fingerprinting”—tracking transmission of genotypes/identifying sources of infection and risk factors	Highly variable genetic markers that are not under selection by the host
Genetic markers/linking phenotype and genotype	Identifying phenotypic traits of clinical significance	Genotype linked to phenotype

molecular techniques (genotypic methods) to detect and characterize the genetic variability of infectious agents (bacteria, fungi, protozoa, viruses) is the foundation for the majority of molecular epidemiological studies. The application of appropriate molecular techniques has been an aid in the surveillance of infectious agents and in determining sources of infection. These molecular techniques can be used to study health and disease determinants in animal (including human) as well as plant populations. It requires choosing a molecular method(s) that is capable of discriminating genetic variants at different hierarchical levels, coupled with the selection of a region of nucleic acid, which is appropriate to the questions being asked (Table 8.7).

Genotypic methods are those that are based on an analysis of the genetic structure of an organism. Over the past decade, a number of genotypic methods have been used to fingerprint pathogenic microorganisms (Table 8.8). The methods have been described in detail elsewhere.^{23–27} Among these methods, RFLP-PFGE (restriction fragment length polymorphism/pulsed-field gel electrophoresis) and RFLP + probe, and ribotyping have been the most commonly used methods for fingerprinting bacteria.^{25,28} RAPD (random amplification of polymorphic DNA) and karyotyping have been used for fingerprinting fungi.^{25,29} MLEE (multilocus enzyme electrophoresis), RAPD, and PCR (polymerase chain reaction)-RFLP have been used for fingerprinting parasitic protozoa.²⁵ Select gene or complete genome characterization, as well as other molecular methods, have been used for viruses.³⁰

When should fingerprinting be used? Strain typing data are most effective when they are collected, analyzed, and integrated into the results of an epidemiological investigation. The epidemiologist should consult the laboratory

TABLE 8.8 Examples of genotypic methods used in epidemiologic investigations

Restriction endonuclease-based methods

- A. Restriction fragment length polymorphism (RFLP) without hybridization
 - Frequent cutter (4–6bp recognition site) coupled with conventional electrophoresis to separate restriction fragments
 - Infrequent cutter (generally 6–8bp recognition site) coupled with pulsed-field gel electrophoresis (PFGE) to separate restriction fragments
- B. RFLP with hybridization
 - Frequent cutter (4–6bp recognition site) coupled with conventional electrophoresis to separate restriction fragments followed by Southern transfer to nylon membrane. The power and efficacy of typing method depends on the probe.
 - 16S and 23S rRNA (ribotyping)
 - Insertion sequences (e.g., IS6110 of *Mycobacterium tuberculosis*)

Amplification-based methods

- A. Random amplification of polymorphic DNA (RAPD) analysis; arbitrarily primed PCR (APPCR)
- B. Amplified fragment length polymorphism (AFLP) method
- C. Repetitive element PCR (REP-PCR) method; variable number tandem repeat (VNTR) fingerprinting

Sequence-based methods

- A. Multilocus sequence typing (MLST)
 - B. Electrophoretic karyotyping
-

when investigating a potential outbreak of an infectious disease. Microbial fingerprinting should supplement, and not replace, a carefully conducted epidemiological investigation. In some cases, typing data can effectively rule out an outbreak and thus avoid the need for an extensive epidemiological investigation. In other cases, these data may reveal the presence of outbreaks caused by more than one strain. Data interpretation is facilitated greatly by an appreciation of the molecular basis of genetic variability of the organism being typed and the technical factors that can affect results. With the exception of whole-genome sequencing, the molecular methods analyze only a small portion of the organisms' genetic complement. Thus, isolates that give identical results are classified as "indistinguishable," not "identical." Theoretically, a more detailed analysis should uncover differences in the isolates that appeared to give identical patterns, but that were epidemiologically unrelated. This is unlikely to occur when a set of epidemiologically linked isolates are analyzed.²³ For this reason, only whole-genome sequencing would provide the unequivocal data required for attribution.

The power of molecular techniques in epidemiological investigations is well exemplified by a few examples. PulseNet, the national molecular subtyping network for foodborne disease surveillance, was established by the CDC and

several state health departments in 1996 to facilitate subtyping bacterial foodborne pathogens for epidemiologic purposes. Twenty years ago, most foodborne outbreaks were local problems that typically resulted from improper food-handling practices. Outbreaks were often associated with individual restaurants or social events, and often came to the attention of local public health officials through calls from affected persons. Today, foodborne disease outbreaks commonly involve widely distributed food products that are contaminated before distribution, resulting in cases that are spread over several states or countries. The PulseNet network, which began with 10 laboratories typing a single pathogen (*Escherichia coli* O157:H7), has grown and now includes 46 state and two local public health laboratories and the food safety laboratories of the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA).²⁸ Currently, four foodborne pathogens (*E. coli* O157:H7, nontyphoidal *Salmonella* serotypes, *Listeria monocytogenes*, and *Shigella*) are being subtyped by PFGE as part of routine surveillance for foodborne disease. The laboratories follow a standardized protocol using similar equipment so that results are highly reproducible and DNA patterns generated at different laboratories can be compared. Isolates are subtyped on a routine basis, and the data analyzed promptly at the local level. Clusters can often be detected locally that could not have been identified by traditional epidemiologic methods alone. PFGE patterns are shared between participating laboratories electronically, which serves to link apparently unrelated outbreaks and facilitates the identification of a common vehicle.³¹ For example, in May 1998, PulseNet facilitated the investigation of two clusters of *E. coli* O157:H7 in the northeastern U.S. PFGE fingerprinting of the *E. coli* O157:H7 isolates by the PulseNet laboratories in that region revealed two simultaneous clusters of *E. coli* O157:H7 infections (32 isolates in four of five states with one PFGE pattern, and 25 isolates in all five states with a second pattern), one of which could be traced to two supermarkets that received ground beef from the same distributor. Without molecular typing, epidemiologists would have found it difficult to identify cases associated with each cluster. On the other hand, the use of PFGE subtyping as part of routine surveillance has benefits beyond outbreak detection. For example, the temporal clustering of unrelated cases is not uncommon, and without molecular typing, valuable public health resources would be wasted investigating pseudo-outbreaks.

Another example of the power of molecular techniques in solving an epidemiologic investigation involves a case of HIV transmission by a healthcare worker. The investigation involved a young woman who had contracted AIDS even though she had no identifiable risk factors. During the investigation, it was revealed that 2 years previously she had several teeth extracted by a dentist who was subsequently confirmed as having AIDS. A retrospective case-control study was conducted of the dentist and his former patients to evaluate the pos-

sibility of dentist-to-patient transmission. Patients were questioned to ascertain known risk factors for HIV transmission. Infection control practices in the dental office were also evaluated.³² Eight HIV-positive persons were identified from among a group of more than 1,000 former patients of the dentist. Five of the eight patients had no risk factors or other documented exposures to HIV. Although all five had undergone invasive procedures, and four of the five shared visit days, no identifiable mechanism of transmission could be established by traditional case-control methodology.³² However, a comparison of the nucleotide sequences of several regions of the gp120 gene of the HIV strains of the dentist, HIV-positive patients (with and without known risk factors), and 35 HIV-infected community controls established the likelihood of a common source of infection.³³ The genetic distance of viruses from the five patients without known risk factors and the virus from the dentist was 3.4%–4.9%, which is similar to that found previously with HIV viruses from persons with epidemiologically linked infections. In contrast, isolates from patients with known risk factors were more distantly related (>10%) to the HIV virus obtained from the dentist.³³ The average genetic distance of viruses from the five patients and the community controls was approximately 11%, which was virtually identical to the average distance among the 35 HIV viruses from controls. Phylogenetic tree analysis confirmed that the HIV viruses from the dentist and the five patients formed a tightly related cluster.³³

SUMMARY

With few exceptions, it will require a careful epidemiologic investigation to determine whether an outbreak of infectious disease is due to the intentional release of an agent, or is naturally occurring. A number of molecular techniques have been developed for subtyping bacteria, viruses, fungi, and protozoa, which will facilitate this investigation as well as identify clusters of related microorganisms.

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