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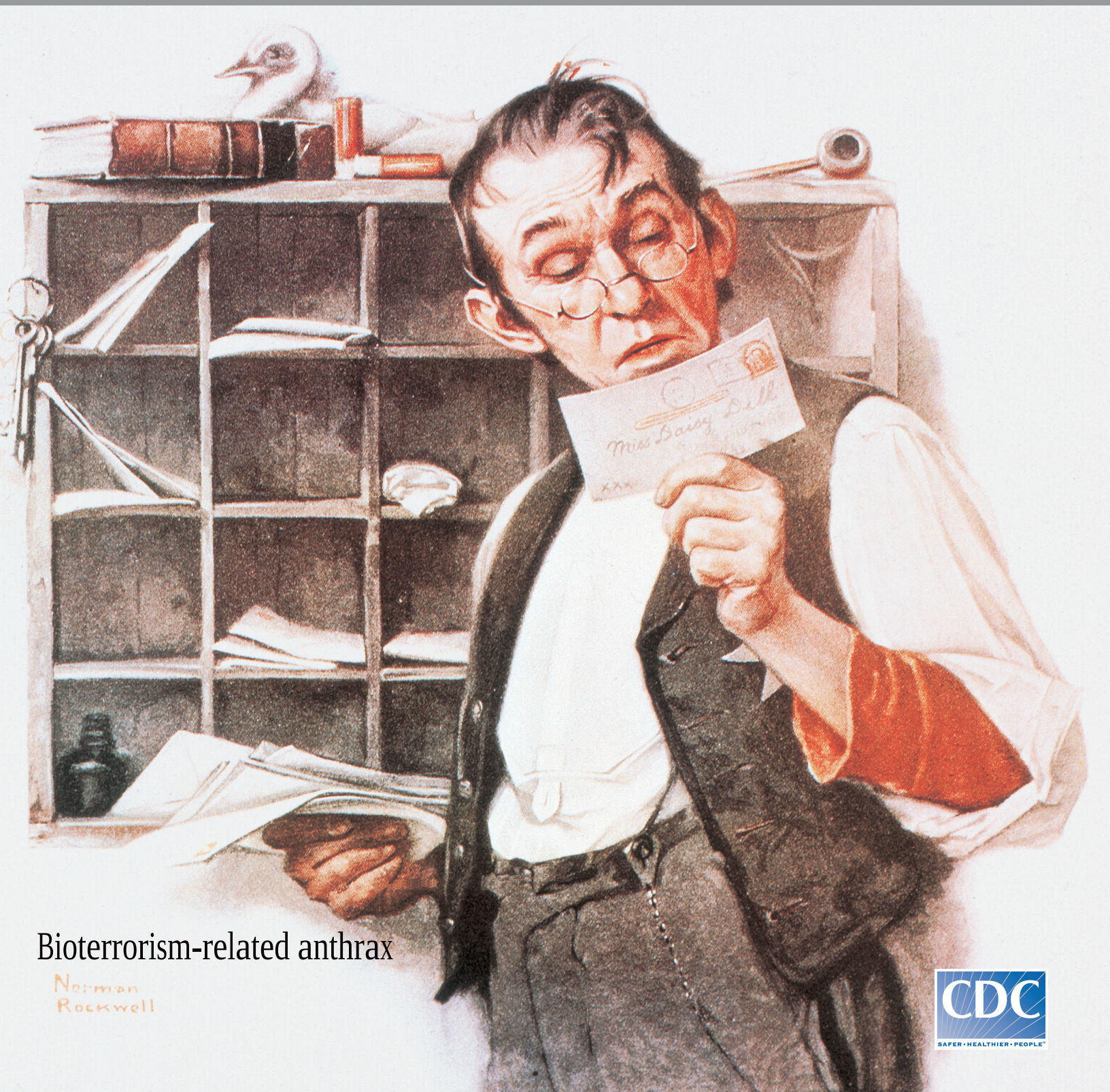

Bioterrorism-related anthrax

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EMERGING INFECTIOUS DISEASES

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Anthrax Bioterrorism: Lessons Learned and Future Directions

James M. Hughes* and Julie Louise Gerberding*

James M. Hughes

Julie L. Gerberding

On September 11, 2001, the United States experienced the worst terrorist attack in its history. As the nation sought to deal with this tragedy, it would face a second wave of terrorism—this time, in the form of a biological attack. The suspicion of anthrax in a patient by an astute infectious disease clinician along with capable clinical and public health laboratory staff in Florida would lead to the discovery that *Bacillus anthracis* spores had been intentionally distributed through the postal system, causing 22 cases of anthrax, including 5 deaths, and forever changing the realm of public health.

In this issue of Emerging Infectious Diseases, numerous individuals involved in the public health aspect of the anthrax investigation document their experiences. Articles describe the epidemiologic and laboratory investigations, applied research findings, environmental assessment and remediation experiences, workplace safety issues, prophylaxis and clinical care information, international aspects, and collaborations between law enforcement and public health officials. The articles also highlight the widespread efforts made to identify the source of exposure and prevent illness among those exposed. While many of the individuals involved in this effort are acknowledged in these articles, many others are not, including the large numbers of medical, public health, law enforcement, and emergency response personnel throughout the country and the world who dealt with the numerous hoaxes perpetrated in the weeks following the attack. We recognize and thank them for their heroic efforts.

This issue also provides an opportunity to review the valuable lessons we have learned from these experiences. Foremost among them is the knowledge that we cannot afford to be complacent. Throughout the Department of Health and Human Services (DHHS) as well as across other federal, state, and local agencies, we remain alert for the first evidence of a disease outbreak. Multiple systems are now in place, both in the United States and internationally, to detect initial cases. On the local level, clinicians and laboratorians play a key role in this process. Activities such as monitoring emergency room visits, pharmacy requests, calls to emergency response and poison control centers, and animal disease registries for unusual occurrences are also expanding.

These lessons have also led us at the Centers for Disease Control and Prevention (CDC) to change the way we operate. Changes have been made within our programs, among our staff and partners, and in our coordination with other federal agencies. Many of these changes have been based on valuable input provided by public and private sector experts during numerous consultations. Terrorism response capacity is being integrated into existing infrastructures, further strengthening the foundation of public health.

The anthrax cases highlighted the importance of the "golden triangle" of response between clinicians and clinical microbiologists, the health-care delivery system, and public health officials. Steps have been taken to strengthen these and other critical linkages, including those between professionals in the human, veterinary, and public health communities and between the public health, law enforcement, and emergency response systems.

DHHS has made available through CDC more than $918 million for state and local health departments to enhance their terrorism preparedness programs. These funds are intended to strengthen capacity to respond to bioterrorism, other infectious disease emergencies, and other urgent public health threats. Existing programs that proved invaluable during the events of last fall, such as the Laboratory Response Network for Bioterrorism (LRN) and the National Pharmaceutical Stockpile (NPS), both described in this issue in the article by Perkins et al., have also been strengthened. During the anthrax attacks, laboratories within the LRN tested more than 125,000 clinical specimens and approximately 1 million environmental specimens. The number of these specialty laboratories participating in this network has now increased to more than 100, with at least one in each state, enabling widespread testing for microbes that might be used in a terrorist attack to cause illnesses such as anthrax, tularemia, plague, and botulism. New facilities have been opened, and improvements in others are in progress or planned for the near future. The NPS has also been

*Centers for Disease Control and Prevention

expanded to include additional medical supplies and personnel. State and local agencies are implementing measures to ensure the successful transport and delivery of these critical components of effective response.

CDC has established rapid response teams composed of individuals with expertise in field operations, epidemiology, microbiology, data management, and communications. These individuals have received training to enable immediate deployment to affected areas to assist state and local efforts. The Epidemic Intelligence Service (EIS), CDC's long-standing disease investigation training program for epidemiologists, is also undergoing changes. In addition to traditional training for rapid response to disease outbreaks, this year's class of officers, the largest in the program's 51-year history, is receiving specialized field training to respond to terrorist attacks that might involve the intentional release of toxic chemicals or spread of infectious agents.

While the terrorist attacks experienced by the United States have enabled us to better prepare for, recognize, and respond to future attacks, more work needs to be done. The anthrax attack was relatively small and did not involve the use of multiple agents, multiple modes of transmission, a drug-resistant organism, transmission to animals, or global spread. The surge capacity of the health-care delivery system was not challenged. In addition, unlike some of the other threat agents, the causative organism was easily isolated in clinical laboratories; there was no risk of person-to-person transmission and no risk of vector-borne transmission.

Planning and practice are essential to ensure an effective response to urgent public health threats. CDC has activated its emergency operations center in response to the recent outbreak of West Nile virus. During 2002, through mid-September, West Nile virus has been identified in more than 40 states and the District of Columbia and has caused more than 1,700 human cases, including more than 80 deaths. Although West Nile virus is a naturally occurring disease, because of its recent arrival in the United States many physicians are unfamiliar with the signs and symptoms suggestive of infection. As part of this response, we have provided professional education to health-care workers, evaluated the quality of laboratory processing of suspected samples, and streamlined communication—all critical components for responding to this outbreak and for identifying ways to improve our capabilities for addressing future emergencies.

Integral to planning is education. Health-care workers, particularly physicians and nurses, need training about the clinical aspects of diseases that may result from the use of biological agents. As has been evident in many recent investigations (e.g., hantavirus pulmonary syndrome, West Nile virus meningitis/encephalitis, anthrax), alert and knowledgeable clinicians and laboratorians are vital to disease surveillance efforts and recognition of new diseases and syndromes. Education of the public regarding the signs and symptoms of diseases associated with infectious agents is also essential. CDC will continue to work with partners in clinical medicine and public health to provide training for health-care providers and microbiologists and to seek innovative ways to disseminate information to the public.

The efforts of this past year to improve terrorism response capacities have been widespread, crossing multiple levels and types of organizations and professions as well as international borders. Within the public health system, we intend to continue these efforts, strengthening existing and establishing new partnerships with diverse agencies, specialties, and disciplines. While we believe that these efforts will enable us to respond aggressively and effectively in the event of a future bioterrorist attack, we acknowledge that inherent to terrorism is the unknown. As was evident in the anthrax investigation, we must learn as we go, adapting our responses as new information becomes available and continuing to strive for excellence in our science, service, systems, and strategies. Investments made in the public health system to increase preparedness to address the threat of bioterrorism will also pay dividends in preparedness to confront the next influenza pandemic, other emerging infectious diseases, and other threats to public health.

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Public Health in the Time of Bioterrorism

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On Thursday, October 4, 2001, just 24 days after the tragic events of September 11, the Florida Department of Health and the Centers for Disease Control and Prevention (CDC) confirmed the first case of inhalational anthrax in the United States in more than 25 years. Recognition of this unexpected case is attributed to the alertness of local infectious disease physician Larry Bush, who promptly notified Jean Malecki, director, Palm Beach County Health Department (1,2). By Saturday, October 6, a team of federal, state, and local public health and local law enforcement investigators identified intentional *Bacillus anthracis* spore contamination at the patient's workplace. These events marked the beginning of the first U.S. outbreak of bioterrorism-related anthrax and (for many of us in clinical medicine, public health, and law enforcement) ushered in the transition from tabletop bioterrorism exercises to real-world investigation and response.

Contingency plans to mitigate bioterrorism-related anthrax outbreaks go back to August 1998, when CDC hosted the "Workshop on Improving Public Health Response to Possible Acts of Bioterrorism." This workshop brought together state and local health departments, public health professional organizations, the U.S. Department of Defense, and the U.S. Department of Justice to examine ways of improving public health preparedness for bioterrorism (CDC, unpub. data). Two investments made as a result of this workshop were the Laboratory Response Network for Bioterrorism and the National Pharmaceutical Stockpile. These early investments were key components of the public health response to the 2001 bioterrorism-related anthrax outbreak.

The Laboratory Response Network was created at the recommendation of the 1998 Workshop's "Diagnosis Working Group," the then Association of State and Territorial Public Health Laboratories (now Association for Public Health Laboratories), and CDC. The Laboratory Response Network is a tiered system of laboratories with capacities defined in an A (lowest tier) through D (highest) pyramid structure (3,4). In support of this structure, procedures for identification of *B. anthracis*, and other Category A biologic agents, were validated, and in some instances developed (or redeveloped) de novo on the basis of older methods. Protocols were written into standard laboratory procedure manuals. Reagents for testing were standardized, produced, and distributed by CDC to participating laboratories. State health department laboratory scientists were trained to use these methods for identifying *B. anthracis*, *Yersinia pestis* (causative agent of plague), and *Francisella tularensis* (causative agent of tularemia) in the fall and winter of 2000. Capacity for specialized or more developmental diagnostic and other tests for *B. anthracis* (e.g., real time polymerase chain reaction [PCR] [5], direct fluorescent-antibody assay [6], immunohistochemical testing, molecular subtyping [7], and antimicrobial susceptibility testing [8]) were established at CDC and (in some instances) at a small number of other advanced U.S. laboratories (e.g., U.S. Army Medical Research Institute of Infectious Diseases, Fort

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Dr. Perkins is chief, Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention (CDC), which has technical responsibility for the CDC epidemiologic and laboratory aspects of *Bacillus anthracis*, and selected other bacterial agents of public health importance. Dr. Perkins led the field team in the investigation of the index case of inhalational anthrax in Florida and participated broadly in the 2001 anthrax investigation and response. His research interests include vaccine evaluation, bacterial meningitis, bioterrorism and emerging infectious diseases. He has worked extensively on the control and prevention of meningococcal disease in the United States, Africa, and around the globe.

Tanja Popovic, Guest Editor
Dr. Popovic is chief, Epidemiologic Investigations Laboratory, Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention. As the subject matter expert on laboratory aspects of *B. anthracis* and anthrax at CDC, she and her staff trained laboratory scientists in all 50 states to isolate and identify *B. anthracis* using standard methodologies in the fall of 2000, and have performed thousands of tests for isolation of *B. anthracis*, its confirmatory identification and molecular subtyping during the 2001 anthrax investigation. In addition to bioterrorism preparedness and response, her research focuses on laboratory diagnosis and molecular epidemiology of bacterial meningitis and diphtheria.

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Dr. Yeskey is director, Bioterrorism Preparedness and Response Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). He has served as deputy director of Emergency Public Health in the Division of Emergency and Environmental Health Services, National Center for Environmental Health, CDC. His previous assignments include associate professor and vice chair, Department of Military and Emergency Medicine, Uniformed Services University School of Medicine, and chief medical officer, U.S. Public Health Service Office of Emergency Preparedness. Dr. Yeskey's experience with disaster response includes work on hurricanes, earthquakes, floods, mass migrations, and terrorist bombings.

Detrick, Frederick, Maryland; Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona). For serologic testing, which was found to be invaluable in identifying anthrax cases during the investigation, existing tests developed for vaccine evaluation were adapted for diagnostic purposes (9). All these laboratory measures were in place before the 2001 anthrax outbreak.

During the acute phase of the outbreak, Laboratory Response Network laboratories processed >121,700 specimens for *B. anthracis* (the bulk from environmental specimens from areas of suspected or confirmed contamination). Public Health Laboratories (other than CDC) tested 84,000 (69%) specimens; the Department of Defense tested 30,200 (25%) specimens; and CDC tested 7,500 (6%) (CDC, unpub. data). Handling the unusual surge of demand without the support of the Laboratory Response Network is difficult to imagine and would have likely compromised the investigation.

The National Pharmaceutical Stockpile was another investment made as a result of the 1998 Workshop and put in place before the 2001 outbreak. During the outbreak, the pharmaceutical stockpile team transported not only antibiotics, anthrax vaccine, clinical and environmental samples, and *B. anthracis* isolates but also epidemiologists, laboratory scientists, pathologists, and specialized teams of researchers. Under extreme pressure, the team made 143 sorties to 9 states and delivered 3.75 million antibiotic tablets from October 8, 2001 to January 11, 2002 (CDC, NPS Program Logistics Log, Oct 2001–Jan 2002).

Other earlier public health investments that paid off during the anthrax outbreak investigation were CDC's more than 50-year-old applied epidemiology training program, Epidemic Intelligence Service, and other academic, state and local health department, and CDC efforts to develop the seasoned cadre of field epidemiologists (10,11) that make up the core of public health investigation and response. These epidemiologists, who work in established networks and make up and often lead complex partnerships, comprise the public health front lines of the bioterrorism response team.

The complexity of the 2001 anthrax investigation and response challenged even experienced field epidemiologists. At the state and federal levels, "incident command"-style management structures were used to address the constant emergence of new information, pursue many public health activities simultaneously across multiple investigations, and communicate effectively. These management structures, which have been adopted by the disaster management and law enforcement communities, are less familiar to public health workers. With some variation from site to site, a typical field investigation structure included local, state, and federal public health partners working on the following teams: Epidemiologic Investigation (what happened?), Intervention (post-exposure prophylaxis and follow-up), Surveillance (identify additional cases), Clinical Evaluation (rapidly evaluate suspect cases), Environmental Assessment (environmental sampling and processing), Remediation (working with the Environmental Protection Agency), and Communication (with the public, partners, and press). These teams were sometimes complemented with Federal Bureau of Investigation (FBI) liaisons; in some cases, public health officials were assigned to FBI investigation teams (12). A senior epidemiologist was also posted to FBI Headquarters in Washington, D.C.

After the October 12 recognition of cutaneous anthrax in New York (13), an emergency operations center was established at CDC, Atlanta, Georgia, to coordinate the outbreak investigation and response. The center tasked more than 2,000 employees (in the field or at headquarters in Atlanta) (CDC unpub. data) to specific functions, including 24-hour response capacity with telephone information and call-triage services and other specialized teams (14). CDC/Atlanta-based teams led by senior epidemiologists supported each field investigation team in involved jurisdictions (Florida, New York, Washington D.C., New Jersey, and Connecticut). These teams were in direct and frequent communication with their respective field team about laboratory results, other investigations, and policy decisions. Other teams included the following: Clinical Medicine (evaluation of suspected cases, post-exposure prophylaxis and treatment recommendations) (15–21); Environmental Assessment (evaluation of suspected or confirmed areas of environmental contamination); International Support (22,23); Laboratory Support (coordination across CDC laboratories and the Laboratory Response Network); National Pharmaceutical Stockpile (antibiotics, vaccine, specimens, and people transport); Postal Service Liaison (partnership with the U.S. Postal Service—CDC also assigned a senior epidemiologist to the Postal Service); and State Liaison (to coordinate requests from states without confirmed anthrax cases) (24). Beginning on October 12, CDC's Morbidity and Mortality Weekly Report published a series of reports, notices, and guidelines as events unfolded (25).

Many unknowns confronted the public-health response team during the anthrax investigation (26). The basics about exposure to *B. anthracis*–contaminated envelopes specifically sent to media outlets and government leaders were understood quickly, given the events in Florida, New York, and then Washington, D.C. (13). Difficulties arose in characterizing anthrax risk to individuals and groups with suspected or confirmed exposure to *B. anthracis*–contaminated envelopes or environments (27). Challenges also arose in the evaluation of *B. anthracis*-containing powders, epidemiologic investigation (28), environmental assessment (29,30) and remediation, surveillance (31,32), diagnosis, treatment, and post-exposure prophylaxis (33–35).

Work with *B. anthracis*–contaminated goat hair in textile mills more than 40 years ago provided some data about the risk of *B. anthracis* spore-containing particles in naturally contaminated occupational environments. These data suggested that relatively high levels of *B. anthracis* spores were "not necessarily or consistently dangerous" in this setting (36). Biologic warfare experts considered it unlikely that terrorists could produce a *B. anthracis* spore powder for use in an

envelope that would be capable of generating substantial primary (or secondary) aerosol threats for human infection or widespread contamination of environments. Yet, in Senator Daschle's office, in the Hart Senate Office building, in the room where the letter was opened (as well as outside the room) exposed persons' nasal mucosa were almost immediately contaminated (37). Re-aerosolization (secondary aerosol) at a level consistent with potential transmission was demonstrated off the implicated high-speed sorter in the Brentwood Processing and Distribution Facility (38). Recent research using simulates of *B. anthracis* spores from the Canadian Defense Establishment Suffield suggests that contaminated envelopes can cause heavy aerosol contamination (39). New understanding is accumulating, and this should improve public health response in the future.

The decision-making involved in closing the U.S. Postal Service's Brentwood Processing and Distribution Facility, Washington, D.C., has been criticized. The risk to Brentwood facility employees by contaminated envelopes in transit was not recognized in time to prevent illness in four employees, two of whom died (40). Decisions concerning the Brentwood facility were based on epidemiologic observations in Florida and New York, where no disease occurred among postal workers. A possible explanation for the differential risk is that the *B. anthracis* spore preparation in the October 9 envelopes had a higher potential for aerosolization than the preparation in the September 18 envelopes or that the two mailings were made under or exposed to different environmental conditions (e.g., amount of moisture) that created a different potential for aerosolization. A different aerosolization potential is supported by the epidemic curve in the manuscript by Jernigan et al. (13), which shows a higher proportion of inhalational (versus cutaneous) anthrax cases associated with the October 9 mailing. In naturally occurring disease, once risk is understood, it generally remains constant; however, in intentional contamination, risk may be altered by the perpetrator(s).

During the anthrax investigation, the public health response team was better prepared in some areas than in others. Five deaths were not prevented, but widespread illness and death was averted through early recognition of threats and prompt intervention. We applied what we knew and learned what we did not know. We gained new appreciation for communication and partnerships. For the first time, on November 8, 2001, a sitting President of the United States of America, George W. Bush, visited CDC to support the efforts of public health professionals and others who participated in the anthrax investigation and response. Leaders and individual heroes rose in the ranks of public health, clinical medicine, and law enforcement (41). The substantial role of public health in the 2001 anthrax investigation and response suggests that strong public health infrastructure supported by applied public health and basic-science research are key elements to the control and prevention of future bioterrorism threats.

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References

1. Traeger MS, Wiersma ST, Rosenstein NE, Malecki JM, Shepard CW, Raghunathan PL, et al. First case of bioterrorism-related inhalational anthrax in the United States, Palm Beach County, Florida, 2001. Emerg Infect Dis 2002;8;1029–34.
2. Maillard J-M, Fischer M, McKee KT Jr, Turner LF, Cline JS. First case of bioterrorism-related inhalational anthrax, Florida, 2001: North Carolina investigation. Emerg Infect Dis 2002;8;1035–8.
3. Centers for Disease Control and Prevention. Biological and chemical terrorism: strategic plan for preparedness and response MMWR Morb Mortal Wkly Rep 2000;49:1–26.
4. Khan AS, Morse S, Lillibridge S. Public-health preparedness for biologic terrorism in the USA. Lancet 2000;356:1179–82
5. Hoffmaster AR, Meyer RF, Bowen M, Marston CK, Weyant RS, Barnett GA, et al. Evaluation and validation of a real-time polymerase chain reaction assay for rapid identification of *Bacillus anthracis*. Emerg Infect Dis 2002;8;1178–82.
6. De BK, Bragg SL, Sanden GN, Wilson KE, Diem LA, Marston CK, et al. A two-component direct fluorescent-antibody assay for rapid identification of *Bacillus anthracis*. Emerg Infect Dis 2002;8;1060–5.
7. Hoffmaster AR, Fitzgerald CC, Ribot E, Mayer LW, Popovic T. Molecular subtyping of *Bacillus anthracis* and the 2001 bioterrorism-associated anthrax outbreak, United States. Emerg Infect Dis 2002;8;1111–6.
8. Mohammed MJ, Marston CK, Popovic T, Weyant RS, Tenover FC. Antimicrobial susceptibility testing of *Bacillus anthracis*: comparison of results obtained by using the National Committee for Clinical Laboratory Standards broth microdilution reference and Etest agar gradient diffusion methods. J Clin Microbiol 2002;40:1902–7.
9. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. Emerg Infect Dis 2002;8;1103–10.
10. Thacker SB, Dannenberg AL, Hamilton DH. Epidemic intelligence service of the Centers for Disease Control and Prevention: 50 years of training and service in applied epidemiology. Am J Epidemiol 2001;154:985–92.
11. Bales ME, Dannenberg AL, Brachman PS, Kaufmann AF, Klatsky PC, Ashford DA. Epidemiologic response to anthrax outbreaks: field investigations, 1950-2001. Emerg Infect Dis 2002;8;1163–74.
12. 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;1152-6.
13. Jernigan DB, Raghunathan PL, Bell BP, Brechner R, Bresnitz EA, Butler JC, et al. Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. Emerg Infect Dis 2002;8;1019–28.
14. Mott JA, Treadwell TA, Hennessy TW, Rosenberg PA, Wolfe MI, Brown CM, et al. Call-tracking data and the public health response to bioterrorism-related anthrax. Emerg Infect Dis 2002;8;1088–92.
15. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
16. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.

17. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
18. Centers for Disease Control and Prevention. Updated recommendations for antimicrobial prophylaxis among asymptomatic pregnant women after exposure to *Bacillus anthracis.* MMWR Morb Mortal Wkly Rep 2001;50:960.
19. Centers for Disease Control and Prevention. Update: interim recommendations for antimicrobial prophylaxis for children and breastfeeding mothers and treatment of children with anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1014–1016.
20. Bell DM, Kozarsky PE, Stephens DS. Clinical issues in the prophylaxis, diagnosis, and treatment of anthrax. Emerg Infect Dis 2002 8:222–5.
21. Barakat LA, Quentzel HL, Jernigan JA, Kirschke DL, Griffith K, Spear SM, et al. Fatal inhalational anthrax in a 94-year-old Connecticut woman. JAMA 2002;287:863–8.
22. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1008–10.
23. Polyak CS, Macy JT, Irizarry-De La Cruz M, Lai JE, McAuliffe JF, Popovic T, et al. Bioterrorism-related anthrax: international response by the Centers for Disease Control and Prevention. Emerg Infect Dis 2002;8;1056–9.
24. Tengelsen L, Hudson R, Barnes S, Hahn C. Coordinated response to possible anthrax contamination, Idaho, 2001. Emerg Infect Dis 2002;8;1093–5.
25. Centers for Disease Control and Prevention. Selected articles. Special issue. MMWR Morb Mortal Wkly Rep 2001; 50:877;889–897;909–919;941–948;960;961;962;973–976;984–986;987–990;991;1008–1010;1014–1016;1049–1051;1051–1054.
26. Perkins BA, Ashford DA. Meeting summary: bioterrorism-related *Bacillus anthracis* public health research priorities. Emerg Infect Dis 2002;8;1183.
27. Centers for Disease Control and Prevention. Interim guidelines for investigation and response to *Bacillus anthracis* exposures. MMWR Morb Mortal Wkly Rep 2001;50:987–90.
28. Greene CM, Reefhuis J, Tan C, Fiore AE, Goldstein S, Beach MJ, et al. Epidemiologic investigations of bioterrorism-related anthrax, New Jersey, 2001. Emerg Infect Dis 2002;8;1048–55.
29. Sanderson WT, Hein MJ, Taylor L, Curwin BD, Kinnes GM, Seitz TA, et al. Surface sampling methods for *Bacillus anthracis* spore contamination. Emerg Infect Dis 2002;8;1145–51.
30. Teshale EH, Painter J, Burr GA, Mead P, Wright SV, Cseh LF, et al. Environmental sampling for spores of *Bacillus anthracis*. Emerg Infect Dis 2002;8;1083–7.
31. Tan CG, Sandhu HS, Crawford DC, Redd SC, Beach MJ, Buehler J, et al. Surveillance for anthrax cases associated with contaminated letters, New Jersey, Delaware, and Pennsylvania, 2001. Emerg Infect Dis 2002;8;1073–7.
32. Williams AA, Parashar UD, Stoica A, Ridzon R, Kirschke DL, Meyer RF, et al. Bioterrorism-related anthrax surveillance, Connecticut, September-December, 2001. Emerg Infect Dis 2002;8;1078–82.
33. Shepard CW, Soriano-Gabarro M, Zell ER, Hayslett J, Lukacs S, Goldstein S, et al. Antimicrobial postexposure prophylaxis for anthrax: adverse events and adherence. Emerg Infect Dis 2002;8;1124–32.
34. Jefferds MD, Laserson K, Fry AM, Roy S, Hayslett J, Grummer-Strawn L, et al. Adherence to antimicrobial inhalational anthrax prophylaxis among postal workers, Washington, D.C., 2001. Emerg Infect Dis 2002;8;1138–44.
35. Williams JL, Noviello SS, Griffith KS, Wurtzel H, Hamborsky J, Perz JF, et al. Anthrax postexposure prophylaxis in postal workers, Connecticut, 2001. Emerg Infect Dis 2002;8;1133–7.
36. Brachman PS, Plotkin SA, Bumford FH, Atchison MM. An epidemic of inhalation anthrax: the first in the twentieth century. Am J Hyg 1960:72:6-23
37. Hsu VP, Lukacs SL, Handzel T, Hayslett J, Harper S, Hales T, et al. Opening a *Bacillus anthracis*-containing envelope, Capitol Hill, Washington, D.C.: the public health response. Emerg Infect Dis 2002;8;1039–43.
38. Dull PM, Wilson KE, Kournikakis B, Whitney EAS, Boulet CA, Ho JYW, et al. *Bacillus anthracis* aerosolization associated with a contaminated mail sorting machine. Emerg Infect Dis 2002;8;1044–7.
39. Kournakakis B., Armour SJ, Boulet CA, Spence M, Barsons B. Risk assessment of anthrax threat letters. DRES Technical Report TR 2001-048. September, 2001.
40. Dewan PK, Fry AM, Laserson K, Tierney BC, Quinn CP, Hayslett JA, et al. Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. Emerg Infect Dis 2002;8;1066–72.
41. Gerberding JL, Hughes JM, Koplan JP. Bioterrorism preparedness and response: clinicians and public health agencies as essential partners. JAMA 2002;287:898–900.

Investigation of Bioterrorism-Related Anthrax, United States, 2001: Epidemiologic Findings

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In October 2001, the first inhalational anthrax case in the United States since 1976 was identified in a media company worker in Florida. A national investigation was initiated to identify additional cases and determine possible exposures to *Bacillus anthracis*. Surveillance was enhanced through health-care facilities, laboratories, and other means to identify cases, which were defined as clinically compatible illness with laboratory-confirmed *B. anthracis* infection. From October 4 to November 20, 2001, 22 cases of anthrax (11 inhalational, 11 cutaneous) were identified; 5 of the inhalational cases were fatal. Twenty (91%) case-patients were either mail handlers or were exposed to worksites where contaminated mail was processed or received. *B. anthracis* isolates from four powder-containing envelopes, 17 specimens from patients, and 106 environmental samples were indistinguishable by molecular subtyping. Illness and death occurred not only at targeted worksites, but also along the path of mail and in other settings. Continued vigilance for cases is needed among health-care providers and members of the public health and law enforcement communities.

In the United States, *Bacillus anthracis* infections have primarily occurred through exposure to infected animals or contaminated animal products such as wool (1). Cases of anthrax have been reported infrequently since the 1970s; the last reported case of inhalational anthrax in the United States occurred in 1976, and the last reported case of cutaneous anthrax occurred in the summer of 2001 (2,3). Outbreaks of inhalational anthrax among humans were linked to occupational exposures at a goat-hair–processing plant in New Hampshire in 1957 and suspected accidental release of *B. anthracis* aerosols from a bioweapons facility in Sverdlovsk, Russia, in 1979 (4,5). Human cases also have occurred in association with large epidemics of anthrax among animals. Because the bacteria can persist for long periods of time as a spore and can be prepared in a powdered formulation, *B. anthracis* has been considered a serious biological threat, with potential use as a military or terrorist weapon (6).

After terrorist attacks on the World Trade Center and the Pentagon in 2001, envelopes containing *B. anthracis* spores were mailed to news media companies and government officials, leading to the first bioterrorism-related cases of anthrax in the United States. We report the combined findings from the epidemiologic and laboratory investigations of these cases, conducted through coordinated efforts of medical and laboratory communities and local, state, and federal public health and law enforcement agencies.

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Methods

Investigators from public health and law enforcement at the federal, state, and local levels collaborated to identify possible cases of anthrax, describe case and exposure characteristics, and prevent further cases through public health interventions. We classified cases as confirmed or suspected on the basis of laboratory and clinical findings (7). A confirmed case of anthrax was defined as clinically compatible illness (cutaneous, inhalational, or gastrointestinal) that was either 1) laboratory confirmed by isolation of *B. anthracis* from a patient's clinical specimens, or 2) associated with other laboratory evidence of *B. anthracis* infection based on at least two supportive tests. A suspected case of anthrax was defined as a clinically compatible illness with no alternative diagnosis and no isolation of *B. anthracis*, but with either 1) laboratory evidence of *B. anthracis* by one supportive laboratory test or 2) an epidemiologic link to an environmental *B. anthracis* exposure.

Laboratory criteria for the case definition of anthrax were 1) isolation of *B. anthracis* from a clinical specimen from a patient's affected tissue or site, with confirmation by direct fluorescent-antibody staining and gamma phage lysis (8); or 2) other supportive laboratory tests, including a) evidence of *B. anthracis* DNA by polymerase chain reaction (PCR) from specimens from a patient's affected tissue or site, b) demonstration of *B. anthracis* in a clinical specimen by immunohistochemical staining (IHC), or c) positive serologic testing by an investigational enzyme-linked immunosorbent assay (ELISA) that determined the concentration of serum immunoglobulin G (IgG) to the protective antigen (PA) component of anthrax toxin; sera were considered reactive if antibody was neutralized by competitive inhibition (9,10).

Case finding was initiated by local, state, and federal public health agencies in all 50 U.S. states and through government agencies in other countries. Hospital- and clinic-based surveillance for possible cases of inhalational anthrax in selected regions was done by provider-based reporting and medical record review of patients seen in emergency departments, intensive-care units, and outpatient clinics and in consultation with dermatologists and other medical specialists. Surveillance was also conducted among medical examiners and at affected news media, government, and postal workplaces. Various electronic communication networks of infectious disease physicians, dermatologists, infection control professionals, emergency department physicians, laboratorians, and others were used to increase awareness among practitioners to recognize and report possible cases of anthrax. Case definitions and characteristics, diagnostic and treatment information, and other findings were communicated through the Centers for Disease Control and Prevention (CDC)'s Morbidity and Mortality Weekly Report, Epidemic Information Exchange, and Health Alert Network.

Investigators responded to reports of possible cases from clinicians, law enforcement officials, and the general public. Possible case-patients or exposed persons were interviewed with site-specific data collection forms. Public health laboratories tested clinical specimens, powder-containing envelopes, and environmental samples for the presence of *B. anthracis*. Demographic data, clinical presentation, exposure risk information, preliminary clinical and environmental laboratory test results, and other findings were collected. Reports of cases meeting the surveillance case definition were forwarded to CDC.

The multistate investigation was conducted by state and local health departments in collaboration with CDC and was coordinated through CDC's Emergency Operations Center (EOC). The EOC, which used an incident command system structure, was organized into teams of epidemiologists, laboratorians, environmental scientists, communication specialists, and logisticians. EOC teams supported local, state, and federal public health investigators in Florida, New York City, New Jersey, the District of Columbia metropolitan area, and Connecticut. A separate EOC team served as a liaison to state health departments and laboratories. Teams also coordinated interactions with the U.S. Postal Service, Department of Defense, Federal Bureau of Investigation, and other federal agencies and organizations. Intervention teams were initiated to coordinate environmental monitoring and decontamination, postexposure prophylaxis and vaccination, and deployment of National Pharmaceutical Stockpile program assets. Reports of cases and environmental sampling, updates of interventions, and other activities were communicated to the EOC for coordinating the investigation and for communications with federal and state partners, and the media.

Environmental investigations were performed at sites possibly contaminated with *B. anthracis* spores to assess the presence and extent of contamination and to guide decontamination and environmental remediation. Environmental samples at news media and postal facilities, residences, and other sites were taken by surface sampling with swabs, wipes, HEPA vacuum filtration, and air sampling (11,12). Nasal swab specimens were collected to define the area of exposure to aerosolized *B. anthracis* and ascertain where a person with inhalational anthrax might have been exposed. Because the sensitivity of nasal swab cultures wanes, attempts were made to obtain cultures within 7 days of exposure. The presence of *B. anthracis* from nasal swab cultures was not determined by Gram stain or colony characteristics alone but required confirmatory testing by qualified laboratories.

Environmental samples were collected by public health, law enforcement, and contract staff and were tested at laboratories participating with the local, state, and federal investigation efforts. Suspect culture colonies were screened by standard Laboratory Response Network Level A testing procedures for identification of *B. anthracis* and confirmed by standard Level B procedures, such as direct fluorescent-antibody staining and gamma phage lysis (8,13). Antimicrobial susceptibility patterns were determined for selected *B. anthracis* isolates by National Committee for Clinical Laboratory Standards (NCCLS) MIC breakpoints for staphylococci (14). NCCLS has not defined either a *B. anthracis* or staphylococcal inter-

pretive breakpoint for ceftriaxone; thus, breakpoints for gram-negative organisms were used to interpret ceftriaxone results. Isolates of *B. anthracis* recovered from clinical specimens, environmental samples, and powder-containing envelopes were subtyped to show genetic relationships by multiple-locus variable-number tandem repeat analysis (MLVA) (15). Statistical analysis of epidemiologic data to calculate measures of association was performed by using EpiInfo (CDC, Atlanta, GA) and SAS (SAS Institute, Inc., Cary, NC).

Results

From October 2 to November 20, 2001, investigators identified 22 cases of bioterrorism-related anthrax; 11 were confirmed as inhalational anthrax and 11 (7 confirmed and 4

Table 1. Demographic, clinical, and exposure characteristics of 22 cases of bioterrorism-related anthrax, United States, 2001

Case no.	Onset date, 2001	Date of anthrax diagnosis by lab testing	State[a]	Age (yrs)	Sex[a]	Race[a]	Occupation[a]	Case status[b]	Anthrax presentation[b]	Outcome	Diagnostic tests[a]
1	9/22	10/19	NY	31	F	W	NY Post employee	Suspect	Cutaneous	Alive	Serum IgG reactive
2	9/25	10/12	NY	38	F	W	NBC anchor assistant	Confirmed	Cutaneous	Alive	Skin biopsy IHC+ / serum IgG reactive
3	9/26	10/18	NJ	39	M	W	USPS machine mechanic	Suspect	Cutaneous	Alive	Serum IgG reactive
4	9/28	10/15	FL	73	M	W, H	AMI mailroom worker	Confirmed	Inhalational	Alive	Pleural biopsy IHC+ / serum IgG reactive
5	9/28	10/18	NJ	45	F	W	USPS mail carrier	Confirmed	Cutaneous	Alive	Skin biopsy IHC+ and PCR+ / serum IgG reac.
6	9/28	10/12	NY	23	F	W	NBC TV news intern	Suspect	Cutaneous	Alive	Serum IgG reactive
7	9/29	10/15	NY	0.6	M	W	Child of ABC employee	Confirmed	Cutaneous	Alive	Skin biopsy IHC+ / blood PCR+
8	9/30	10/4	FL	63	M	W	AMI photo editor	Confirmed	Inhalational	Dead	Cerebrospinal fluid culture +
9	10/1	10/18	NY	27	F	W	CBS anchor assistant	Confirmed	Cutaneous	Alive	Skin biopsy IHC+ / serum IgG reactive
10	10/14	10/19	PA	35	M	W	USPS mail processor	Confirmed	Cutaneous	Alive	Blood culture + / serum IgG reactive
11	10/14	10/28	NJ	56	F	B	USPS mail processor	Confirmed	Inhalational	Alive	Blood PCR+ / pleural fluid cytology IHC+ / serum IgG reactive
12	10/15	10/29	NJ	43	F	A	USPS mail processor	Confirmed	Inhalational	Alive	Pleural fluid IHC+ / bronchial biopsy IHC+ / serum IgG reactive
13	10/16	10/21	VA	56	M	B	USPS mail worker	Confirmed	Inhalational	Alive	Blood culture +
14	10/16	10/23	MD	55	M	B	USPS mail worker	Confirmed	Inhalational	Dead	Blood culture +
15	10/16	10/26	MD	47	M	B	USPS mail worker	Confirmed	Inhalational	Dead	Blood culture +
16	10/16	10/22	MD	56	M	B	USPS mail worker	Confirmed	Inhalational	Alive	Blood culture +
17	10/17	10/29	NJ	51	F	W	Bookkeeper	Confirmed	Cutaneous	Alive	Skin biopsy IHC+ and PCR+ / serum IgG reactive
18	10/19	10/22	NY	34	M	W, H	NY Post mail handler	Suspect	Cutaneous	Alive	Skin biopsy IHC+
19	10/22	10/25	VA	59	M	W	Government mail processor	Confirmed	Inhalational	Alive	Blood culture +
20	10/23	10/28	NY	38	M	W	NY Post employee	Confirmed	Cutaneous	Alive	Skin biopsy culture +
21	10/25	10/30	NY	61	F	A	Hospital supply worker	Confirmed	Inhalational	Dead	Pleural fluid and blood culture +
22	11/14	11/21	CT	94	F	W	Retired at home	Confirmed	Inhalational	Dead	Blood culture +

[a]NY, New York; FL, Florida; NJ, New Jersey; PA, Pennsylvania; VA, Virginia; DC, District of Columbia; MD, Maryland; CT, Connecticut; F, female; M, male; W, white; B, black; A, Asian; W,H, white with Hispanic ethnicity; NBC, National Broadcasting Company; AMI, American Media Inc.; USPS, United States Postal Service; CBS, Columbia Broadcasting System; PCR, polymerase chain reaction; IHC, immunohistochemical staining; + positive; IgG, immunoglobulin G.
[b]Case status and anthrax presentation are described in the anthrax surveillance case definition in the Methods section.

suspected) as cutaneous anthrax. The demographic, clinical, and exposure characteristics of each patient are presented in Table 1. In March 2002, an additional case of cutaneous anthrax was reported in a laboratory worker processing environmental samples of *B. anthracis* in support of the CDC investigation of the fall 2001 bioterrorism-related anthrax attacks (16).

Characteristics of Case-Patients

Cases were identified in residents of seven states along the east coast of the United States: Connecticut, one case; Florida, two cases; Maryland, three; New Jersey, five; New York City, eight (includes a case in a New Jersey resident exposed in New York City); Pennsylvania, one; and Virginia, two. The median age of patients was 46 years (range 7 months to 94 years) (Table 2). Patients with inhalational anthrax were older than those with cutaneous disease (56 vs. 35 years, $p<0.01$). Twelve (55%) patients were male; 15 (68%) were white. Five (23%) case-patients died; deaths occurred only in patients with inhalational anthrax. The case-fatality ratio for inhalational anthrax was 45%. For six cases of inhalational anthrax in postal workers, we were able to estimate the date of first exposure to *B. anthracis*–positive envelopes processed with high-speed sorters. The mean duration between exposure and onset of symptoms of inhalational anthrax in these patients was 4.5 days (range 4–6).

All 11 cases of inhalational anthrax met the surveillance definition for a confirmed case; 8 were confirmed by isolation of *B. anthracis* from a clinical specimen—7 from blood and 1 from cerebrospinal fluid (Table 1). Supportive laboratory tests used to confirm three other cases of inhalational anthrax included IHC or PCR of tissues (pleural biopsy, pleural fluid, or blood) and elevation between acute- and convalescent-phase serum anti-PA IgG by ELISA (9).

Seven (64%) of the 11 cases of cutaneous anthrax met the surveillance definition for a confirmed case; 2 were confirmed by isolation of *B. anthracis* from a clinical specimen, 1 from blood and 1 from a wound (Table 1). Supportive laboratory tests used in the remaining five confirmed cutaneous cases included IHC or PCR of skin biopsies, PCR of blood, and elevation of serum anti-PA IgG by ELISA. Four cutaneous cases each had only one supportive laboratory test for *B. anthracis* infection and were classified as suspected: one case had a positive IHC of a skin biopsy, and three had elevated serum anti-PA IgG by ELISA. Among cutaneous anthrax cases, lesions were distributed on the face, arms, or chest; two cases had multiple lesions.

We classified patients into two broad exposure categories on the basis of their primary job duties (Table 2). Twelve (55%) patients (8 with inhalational and 4 with cutaneous disease) were mail handlers, including U.S. Postal Service employees (9 cases), government mail processing staff (1case), and media company mailroom workers (2 cases). Six (27%) patients (one inhalational and five cutaneous cases) were media company employees working at sites where powder-containing mail was received: American Media, Inc. (AMI), one case; Columbia Broadcasting System (CBS), one case; National Broadcasting Company (NBC), two cases; and New York Post, two cases. Four (18%) case-patients (two inhalational and two cutaneous cases) were classified as "other," including a 7-month-old visitor to the American Broadcasting Company (ABC), a 61-year-old Manhattan hospital supply room worker, a 51-year-old bookkeeper from New Jersey, and a 94-year-old Connecticut resident. For analysis, we excluded case-patients in the "other" category and compared mail handlers with targeted mail recipients. Mail handlers were older ($p<0.01$) and were associated with inhalational disease (odds ratio [OR] 10; 95% confidence intervals [CI] $0.65 < OR < 530.48$; $p=0.13$). Whether age or occupation were important independent factors in becoming infected is unknown. Of all 22 patients, 20 (91%) either handled mail potentially contaminated with *B. anthracis* spores or were exposed to worksites where *B. anthracis*–contaminated mail was processed or received.

Table 2. Comparison of inhalational and cutaneous bioterrorism-related anthrax cases, United States, 2001

Case characteristic	All cases, n=22 (%)	Inhalational cases n=11, (%)	Cutaneous cases n=11, (%)	p value (inhal. vs. cutan.)
Median age (range), years[a]	46 (0.6–94)	56 (43–94)	35 (0.6–51)	<0.01
Male sex (percent)	12 (55)	7 (64)	5 (45)	0.7
Occupation/exposure site[a]				
Mail handler	12 (55)	8 (73)	4 (36)	0.13
Media company employees	6 (27)	1 (9)	5 (45)	
Other	4 (18)	2 (18)	2 (18)	
No./deaths (case-fatality ratio)	5 (23)	5 (45)	0 (0)	0.04
No. of cases following contaminated letters[b]				
September 18 mailing	11 (50)	2 (18)	9 (81)	<0.01
October 9 mailing	8 (36)	7 (64)	1 (9)	

[a]Associations suggest that age and occupation varied between inhalational and cutaneous cases; however, it is uncertain if age or occupation were significant independent factors for having a case of anthrax. Wilcoxon two-sample test for nonparametric data was used. All other measurements used two-sided Fisher's exact test.
[b]Based on documented or presumed paths of contaminated envelopes; excludes three case-patients who could not be linked to a particular mailing.

Clinical and Environmental Laboratory Findings

B. anthracis isolates were collected from four powder-containing envelopes, 17 clinical specimens from case-patients, and 106 environmental samples collected along the mail path of the implicated envelopes in Florida, District of Columbia metropolitan area, New Jersey, New York City, and Connecticut. We compared these isolates by MLVA for molecular typing and found that all isolates tested were indistinguishable (17,18). Isolates also had the same antimicrobial susceptibility patterns (18): all isolates tested were susceptible to penicillin (MIC range ≤0.06 μg/mL–0.12 μg/mL), amoxicillin (MIC ≤0.06 μg/mL), ciprofloxacin (MIC ≤0.06 μg/mL), doxycycline (MIC ≤0.03 μg/mL), chloramphenicol (MIC 4 μg/mL), clindamycin (MIC ≤0.5 μg/mL), tetracycline (MIC 0.06 μg/mL), rifampin (MIC ≤0.5 μg/mL), clarithromycin (MIC 0.25 μg/mL), and vancomycin (MIC 1–2 μg/mL). Isolates were borderline susceptible to azithromycin (MIC 2 μg/mL) and intermediate to erythromycin (MIC 1 μg/mL) and ceftriaxone (MIC 16) (19).

Assessment of Exposures

Onsets of symptoms occurred from September 22 to November 14, 2001 (Figure 1). Two distinct case clusters were separated in time; no cases occurred during a 13-day period between clusters. One case of inhalational anthrax in a resident of Connecticut occurred 20 days after the second case cluster.

Envelopes Containing Spores

Four *B. anthracis*–positive powder-containing envelopes were recovered, and the path of the envelopes through the mail was traced (Figure 2). All four envelopes were standard, prestamped U.S. Postal Service issue. Two of the four envelopes, one addressed to NBC news anchor Tom Brokaw and the other to the editor of the New York Post, both in New York City, were mailed in or around Trenton, New Jersey, and were postmarked September 18, 2001. Both these envelopes contained letters with the phrases, "09-11-01…This is next…Take penacilin [sic] now…" (20). The next two envelopes recovered, one addressed to Senator Tom Daschle and one to Senator Patrick Leahy, both in Washington, D.C., were mailed in or around Trenton and were postmarked October 9, 2001. Each envelope contained a letter with statements such as, "09-11-01…You can not stop us. We have this anthrax. You die now. Are you afraid?" No *B. anthracis*–positive powder-containing envelopes were recovered from other sites in New York City or during investigations in Florida or Connecticut.

The September 18 envelopes were transported through various postal facilities along processing and delivery paths between New Jersey and the intended media company targets in New York City. The implicated envelopes were processed at the U.S. Postal Service Trenton Mail Processing and Distribution Center in Hamilton, New Jersey, and were sent to the Morgan Central Postal Facility in New York City, where they were sorted and delivered. Both these facilities and at least five others in New Jersey affiliated with the Hamilton facility

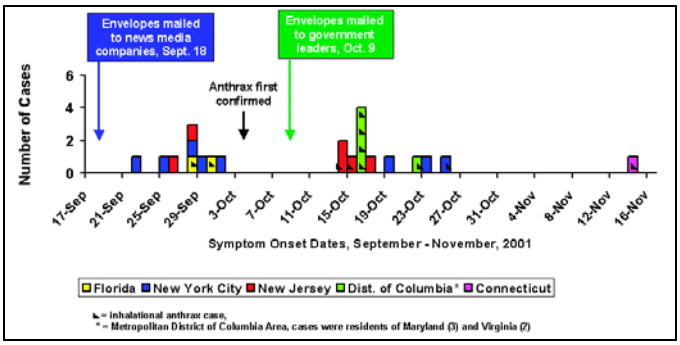

Figure 1. Epidemic curve for 22 cases of bioterrorism-related anthrax, United States, 2001.

had environmental samples positive for *B. anthracis* (21,22). Despite environmental evidence of *B. anthracis* spores at two broadcast media work facilities (ABC, CBS) associated with case-patients, no other *B. anthracis*–positive mail was recovered. Although no *B. anthracis*–positive envelopes were recovered in Florida, *B. anthracis* was isolated from environmental sampling at the AMI building (the worksite of the Florida case-patients) and at least six postal facilities along the path of mail delivered to AMI. The dates of illness onset in AMI media company employees in Florida suggest possible exposure to envelopes mailed in mid-September 2001 (23).

The October 9 envelopes were mailed in or around Trenton, New Jersey, processed at the Hamilton, New Jersey, facility, and transported to the U.S. Postal Service Brentwood Mail Processing and Distribution Center in Washington, D.C. The envelopes were processed with high-speed sorters at both the Hamilton and Brentwood facilities, allowing for the possibility of aerosolized *B. anthracis* spores. The implicated envelopes

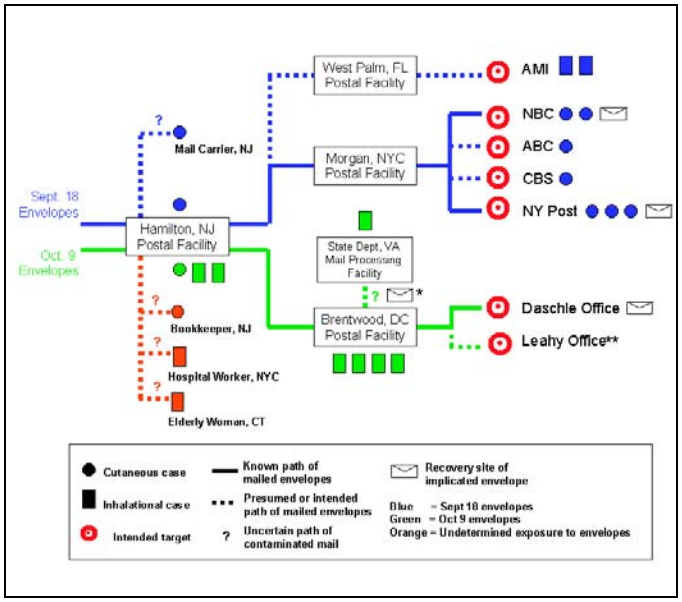

Figure 2. Cases of anthrax associated with mailed paths of implicated envelopes and intended target sites. NY, New York; NBC, National Broadcasting Company; AMI, American Media Inc.; USPS, United States Postal Service; CBS, Columbia Broadcasting System. *Envelope addressed to Senator Leahy, found unopened on November 16, 2001, in a barrel of unopened mail sent to Capitol Hill; **dotted line indicates intended path of envelope addressed to Senator Leahy.

and other subsequently contaminated mail were transported to various government mail facilities. One implicated envelope was delivered to the office of Senator Daschle in the Hart Senate Office Building and was opened by office staff on October 15, 2001. Prompt recognition of the potential for anthrax illness from the powder-containing envelope led to rapid initiation of postexposure chemoprophylaxis for exposed office staff. Beginning October 15, nasal swab specimens were collected from 625 persons potentially exposed at the Hart Senate building to the envelope sent to Senator Daschle on October 9; 28 were found to be positive for *B. anthracis* (24). Environmental sampling showed that sections of the Hart Building and the Brentwood postal facility were heavily contaminated with *B. anthracis* spores. In addition, at least 25 other government, postal, or mail-receiving facilities affiliated with Brentwood had environmental samples positive for *B. anthracis*; some of these facilities did not process the implicated envelopes but received other mail from Brentwood. The other implicated envelope postmarked on October 9, 2001, was addressed to Senator Leahy and was recovered unopened on November 16, 2001, in government mail that had been impounded before delivery to Capitol Hill; the exact delivery path of this envelope is unknown (25).

Case Clusters

The first cluster of nine cases began approximately 4 days after the September 18 envelopes were mailed (Figure 1). All seven cases from New York City and New Jersey in the first case cluster were cutaneous anthrax; all five New York City cases included media company employees or visitors. Both New Jersey cases were in postal employees. The two cases from Florida were both inhalational anthrax and were in media company employees. Overall, eight of the nine persons in the first case cluster were exposed to worksites (postal facilities or media companies) that had environmental samples positive for *B. anthracis*. One case-patient, a New Jersey mail carrier, had no exposure to any contaminated worksite; exposure to *B. anthracis*–positive mail, secondarily contaminated at implicated postal facilities (i.e., cross-contaminated mail), is a likely source of infection. The median number of days from the postmark date of September 18, 2001, to onset of illness in the first case cluster was 10 days (range 4–13 days). Onset of illness for all cases in the first cluster occurred before the first culture identification of *B. anthracis* in the index case of inhalational anthrax in Florida on October 3, 2001 (Figure 1).

The second case cluster began approximately 5 days after the October 9 envelopes were mailed. All five cases from the D.C. metropolitan area were in the second case cluster, all were inhalational anthrax, and all case-patients worked in postal facilities contaminated by the *B. anthracis*–containing October 9 envelopes. The last two cutaneous cases from New York City whose onsets of illness occurred in the second case cluster (cases numbered 18 and 20 in Table 1) were known to have handled the September 18 New York Post envelope when it was moved in mid-October before its identification. Of the four cases from New Jersey in the second cluster, two were inhalational anthrax in postal employees, one was cutaneous anthrax in a postal worker, and one was cutaneous anthrax in a bookkeeper who worked at a nearby commercial office building; all four case-patients were exposed to worksites that had environmental samples positive for *B. anthracis*. No definitive *B. anthracis* exposure was identified for a case of inhalational anthrax in a woman who worked in the supply stockroom of a hospital in Manhattan. Exposure to cross-contaminated mail is a possible source of her infection. The median number of days from the postmark date of October 9, 2001, to onset of illness in the second case cluster was 7 days (range 5–13 days), excluding case-patients with no defined exposure or with exposure to the September 18 envelopes. Thus, the median number of days from mailing of the implicated envelopes to onset of symptoms was an estimated 3 days less for the second cluster; however, there was no statistically significant difference for this comparison.

One case of inhalational anthrax in a 94-year-old female resident of Oxford, Connecticut, had onset of illness on November 14, 2001. No exposure to *B. anthracis* for this patient could be defined, despite extensive environmental sampling at her home and other sites. Environmental samples at the U.S. Postal Service Wallingford Mail Processing and Distribution Center in Wallingford, Connecticut, were positive for *B. anthracis*. The Wallingford facility received mail from the contaminated postal facility in Hamilton, New Jersey, and served as the primary source of mail delivered to the patient's home, suggesting cross-contamination of mail as a possible source of exposure. Postal sorting records indicated that an envelope had been processed in Hamilton on a high-speed sorter 15 seconds after one of the implicated envelopes sent to U.S. senators. That envelope had been delivered to an address 4 miles away from the residence of the Connecticut patient. The envelope was recovered and found to be positive for *B. anthracis*.

We classified cases on the basis of known or likely exposure to contaminated envelopes, accounting for the location, occupation, and estimated incubation period of the case (Table 2). Eleven cases were associated with the September 18 envelopes (case numbers 1–9, 18, and 20; Table 1). Eight cases were associated with the October 9 envelopes (case numbers 10–16, and 19; Table 1). No certain exposure to any implicated envelopes was found for three cases (case numbers 17, 21, and 22; Table 1). Case number 5, a New Jersey mail carrier, had no exposure to the Hamilton facility or any *B. anthracis*–positive worksites; however, we classified this case with the September 18 mailing because onset of illness occurred before the October 9 mailing. When we excluded from analysis the three patients who had no definitive exposures, we found that case-patients associated with the September 18 envelopes were more likely to have been exposed at news media facilities than at postal facilities compared with patients associated with the October 9 envelopes (OR undefined, $p<0.01$). Cases associated with the October 9 envelopes were more likely to be inha-

lational anthrax than were those associated with the September 18 envelopes (OR 31.5; 95% CI 1.76% to 1,570%; p<0.01). These findings suggest that the October 9 mailing was associated with more severe illness and with development of illness following exposures along the path of the mail.

Interventions

Antimicrobial postexposure prophylaxis was recommended for persons at risk for inhalational anthrax given 1) the presence of an inhalational case at a facility (e.g., AMI in Florida), 2) environmental specimens positive for *B. anthracis* in facilities along the path of a contaminated letter where aerosolization might have occurred (e.g., postal facilities in New York City, New Jersey, Connecticut, District of Columbia, and Virginia), and 3) exposure to an air space known to be contaminated with aerosolized *B. anthracis* from an opened letter (e.g., Senate office buildings in the District of Columbia) (26,27). An estimated 32,000 persons initiated antimicrobial prophylaxis; however, completion of a 60-day course of antimicrobial prophylaxis was recommended for approximately 10,300 persons who met the factors listed above (26–28). Because some persons requested additional precautions, especially those exposed to high levels of anthrax spores, more antibiotics—alone or with vaccine—were offered to other persons in the same cohort (29). No additional cases of anthrax have been reported in persons at sites where *B. anthracis* exposures were suspected and where exposed persons initiated antimicrobial prophylaxis. Additional description of antimicrobial postexposure prophylaxis is presented elsewhere (30–32).

Discussion

We identified 22 cases of anthrax that occurred after envelopes containing *B. anthracis*–positive powder were mailed to persons in news media and government. Inhalational and cutaneous disease followed exposure to *B. anthracis* spores; five people died. These cases represent the first reported bioterrorism-related outbreak of anthrax. The investigation of these cases reveals important findings for detecting and preventing infections from bioterrorist attacks.

We tested *B. anthracis* isolates from patients, powder-containing envelopes, and environmental samples from news media, government, and postal processing worksites and found all tested isolates to be indistinguishable by molecular typing methods. Similar U.S. postal service-issue envelopes containing powder preparations of these *B. anthracis* spores were mailed from the Trenton, New Jersey, area on at least two dates. Although isolates, envelopes, and originating postal paths were similar, characteristics of cases differed by date of mailing and geographic region.

Patients in the cluster that occurred after the September 18 mailing were more likely to have cutaneous disease and to have been exposed at news media facilities rather than at postal facilities. Case-patients in the cluster that occurred after the October 9 mailing were more likely to have inhalational disease and to have been exposed at postal facilities along the path of envelopes sent to U.S. senators. Postal workers exposed to *B. anthracis* from the October mailings had predominantly inhalational disease. The case-fatality ratio for all cases of inhalational anthrax was 45%, a ratio lower than previously reported (33); the estimated incubation period of 4.5 days for inhalational cases was consistent with previously reported findings (1).

The fulminant systemic illness associated with the October mailing to U.S. senators differed greatly from the less severe cutaneous cases in media company employees in New York City, suggesting that substantial illness and death likely might have occurred among senate office staff after implicated envelopes were opened. Exposure to *B. anthracis* spores from processing unopened envelopes at the Hamilton and Brentwood postal facilities went unrecognized until after the implicated envelope was opened at the Hart Senate Office Building. Administration of postexposure chemoprophylaxis likely prevented further cases in postal workers and almost certainly averted disease in senate staff. Estimates derived from mathematical models support this conclusion (34). Our findings suggest that prompt use of antimicrobial prophylaxis following suspected bioterrorist attacks can prevent disease.

Differences in the consistency of *B. anthracis* powders between the September and October mailings have been reported by the Federal Bureau of Investigation and may account for the preponderance of inhalational cases in the second cluster (35,36). The later mailings may have intentionally contained a smaller particle-sized powder to produce greater harm. Media company employees had less severe disease than did the postal workers along the path of envelopes sent to senators.

Our findings indicate that the clinical and epidemiologic presentations of a bioterrorist attack depend on the population targeted, the characteristics of the agent, and the mode of transmission. With naturally occurring outbreaks of infection, early cases identified often provide clues to the mode and source of exposure. For bioterrorism-related disease, characteristics of initial cases may be misleading if terrorists vary the mode and source of exposure. Further understanding is needed of the role of different *B. anthracis* powder formulations in the mode of exposure and illness characteristics of persons exposed.

Cases of anthrax occurred in persons near those targeted for infection and also in those along the mail path of spore-containing envelopes. After the mailing of the September 18 envelopes, cases of cutaneous anthrax occurred, but were initially unrecognized, in workers at the postal processing center in New Jersey where the implicated envelopes originated. After the mailing of the October 9 envelopes, inhalational disease was identified in workers at postal facilities in the District of Columbia and New Jersey. Investigators did not anticipate the exposures and fulminant disease in those exposed to aerosols of *B. anthracis* spores from unopened envelopes along the path of the mail. No prior experience with mailed *B. anthracis*–pos-

itive, powder-containing envelopes is described in published reports; previous descriptions of aerosolized *B. anthracis* spores indicated that risk for re-aerosolization or resuspension of spores was low (37). Previous preventive strategies for presumed *B. anthracis* exposures now appear inadequate in light of recent findings. Before this incident, antimicrobial prophylaxis was recommended only for direct exposures to the envelopes, and limited decontamination was suggested only for the immediate site of envelope opening (38). Cutaneous and inhalational disease in postal workers in our investigation clearly shows that sealed, *B. anthracis*–positive, powder-containing envelopes can be a source of infection, presumably via the airborne route, for persons processing contaminated mail in postal facilities. Airborne transmission at the Brentwood and Hamilton facilities may have been facilitated by the use of high-speed sorters, as well as air-blowers used for routine cleaning (12). Any future investigations of bioterrorism-related anthrax should evaluate persons potentially exposed along the path of the delivery vehicle as well as those targeted by the attack.

We found most cases of anthrax to be epidemiologically linked to sites contaminated by implicated envelopes; however, not all cases had direct exposures to targeted worksites, implicated envelopes, or mail-processing facilities along the mail path. Two cutaneous anthrax patients, a mail carrier and a bookkeeper in New Jersey, were not exposed to contaminated postal facilities or media companies. Only one of many environmental samples of surfaces at the bookkeeper's office, where mail was received, was positive for *B. anthracis*. Cross-contaminated mail may be a likely exposure source for anthrax for both these cases.

The possibility of *B. anthracis* exposure from envelopes secondarily contaminated from implicated postal facilities greatly extended the group of potentially exposed persons in our investigation. Experience with anthrax related to agricultural or industrial sources indicated that direct exposure to animals, animal products, and wool-processing facilities accounted for most reported cases (1,3,4,39). Contamination of the environment in animal and wool-processing facilities has been shown, and occasional cases due to secondarily contaminated items have been reported as a possible source of anthrax (1).

For our investigation, contamination found at postal processing facilities off the direct mail path of implicated envelopes indicates that cross-contamination of mail occurred; however, enhanced surveillance for anthrax cases in multiple regions has not identified additional cases. Two patients with inhalational anthrax, a hospital worker in New York City and a retired woman in Connecticut, had no exposure to media or government worksites, implicated postal facilities, or possible sources of naturally occurring anthrax (40). Neither patient had evidence of *B. anthracis* contamination at her home (or workplace for the New York City case), yet both were infected with *B. anthracis* isolates indistinguishable from the outbreak strain. Postal processing facilities in New York City and Wallingford, Connecticut, were contaminated with *B. anthracis*, suggesting cross-contaminated mail as a possible source of *B. anthracis* exposure for both cases.

From our investigation, *B. anthracis*–positive powder appears capable of contaminating other mail during processing, leading to exposure and subsequent development of cutaneous and possibly inhalational anthrax. The risk from cross-contaminated mail appears to be extremely low; 85 million pieces of mail were processed at facilities in New Jersey and District of Columbia after the October 9 envelopes, and no additional anthrax cases were detected through stimulated enhanced hospital-based surveillance of 10.5 million people in metropolitan areas around those postal facilities (41). Although the risk for *B. anthracis* infection from cross-contaminated mail may be low, investigations of future bioterrorist attacks with *B. anthracis*–positive powders should consider the potential role of secondarily contaminated items in transmission of disease. An attack using a greater number of spore-containing envelopes would likely lead to many more cases due to cross-contaminated mail (42).

Throughout the investigation, various reporting mechanisms were used to enhance detection of cases, including prospective syndromic surveillance in emergency departments and intensive-care units, laboratory-based surveillance, networks of clinicians such as dermatologists, and worksite absenteeism monitoring. In general, most cases of anthrax were detected through reports from clinical laboratorians and clinicians and from patient self-reporting. The role of the news media in increasing patient, clinician, and laboratorian awareness of anthrax was likely an important factor in stimulating case detection and reporting. Health departments sent alerts to health-care providers and provided training seminars for clinicians to improve case detection. Before the bioterrorism-related anthrax cases in 2001, clinician recognition of clinical findings suggestive of cutaneous or inhalational anthrax is presumed to have been very low (43,44). For our investigation, cases in the first cluster associated with the September 18 mailing went unrecognized until *B. anthracis* was identified in a culture of cerebrospinal fluid from the index case in Florida, underscoring the critical role of the laboratory in initiating the investigation.

These first unrecognized cutaneous cases demonstrate the potential difficulties in detecting cases from a covert bioterrorism agent release. Once the possibility of anthrax exposures at media companies was recognized, along with subsequent environmental work site samples positive for *B. anthracis*, cases of cutaneous anthrax were more readily detected and reported. During the investigation, rapid dissemination of clinical findings through broadcast e-mail and fax alerts to hospitals and providers, public health reports, and networks of clinical, laboratory, and public health officials provided important tools to frontline clinicians to improve recognition of anthrax. Enhancing the knowledge and skills of clinicians and laboratorians for diagnosing bioterrorism-related infections and improving collaborations between clinicians and public health practitioners

will set the stage for better detection of cases associated with any future acts of bioterrorism.

Our investigation had several limitations. The detection of anthrax cases involved numerous local, state, and federal public health and law enforcement officials. Because of the widely distributed activities of various investigators and the need to act quickly in identifying potential exposure sources, data collection instruments were not uniform. Collation of information across sites was limited to a select set of demographic, exposure, and risk factor data elements. The wide use of postexposure prophylaxis, along with difficulty in obtaining detailed information about potentially exposed persons, prevented general estimates of anthrax attack rates for many sites. Surveillance case definitions required laboratory confirmation of disease or of environmental exposure and thus may have missed cases of disease that were treated empirically without appropriate cultures (e.g., illness empirically treated as infected spider bites, which was actually cutaneous anthrax). Environmental sampling of potentially contaminated facilities used different testing methods; because less sensitive testing methods were used, certain sites may have underrepresented the degree of contamination. Throughout the investigation, there was a continuing need to refine study methods and redetermine intervention recommendations, since prior experience with bioterrorism-related anthrax was lacking. Finally, because the public health investigation was also a criminal investigation, information that may have contributed epidemiologic information may not have been available to many public health investigators because it was protected for use in prosecution.

The attacks initiated response activities in all states across the United States and in other countries and required considerable resources to support investigative efforts at the local, state, and federal levels. The perpetrator has not been apprehended, and new cases can still occur. Continued collaboration with law enforcement officials is required, and clinicians, laboratorians, public health officials, and the general public should remain alert for patient symptoms or findings that might indicate additional cases of bioterrorism-related anthrax.

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References

1. Brachman P, Kaufmann A. Anthrax. In: Evans A, Brachman P, editors. Bacterial infections of humans. New York: Plenum Medical Book Company; 1998. p. 95–107.
2. Suffin SC, Carnes WH, Kaufmann AF. Inhalation anthrax in a home craftsman. Hum Pathol 1978;9:594–7.
3. Centers for Disease Control and Prevention. Human anthrax associated with an epizootic among livestock—North Dakota, 2000. MMWR Morb Mortal Wkly Rep 2001;50:677–80.
4. Plotkin SA, Brachman PS, Utell M, Bumford FH, Atchison MM. An epidemic of inhalation anthrax, the first in the twentieth century: I. Clinical features. This article was originally published in Am J Med 1960;29:992–1001. Am J Med 2002;112:4–12.
5. Meselson M, Guillemin J, Hugh-Jones M, Langmuir A, Popova I, Shelokov A, et al. The Sverdlovsk anthrax outbreak of 1979. Science 1994;266:1202–7.
6. Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, et al. Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. JAMA 1999;281:1735–45.
7. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–93.
8. Turnbull PC. Definitive identification of *Bacillus anthracis*—a review. J Appl Microbiol 1999;87:237–40.
9. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. Emerg Infect Dis 2002;8;1103–10.
10. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
11. Centers for Disease Control and Prevention. Procedures for collecting surface environmental samples for culturing *Bacillus anthracis*. Available from: URL: http://www.bt.cdc.gov/DocumentsApp/Anthrax/11132001/final42.asp . Accessed November 2001.
12. Centers for Disease Control and Prevention. Evaluation of *Bacillus anthracis* contamination inside the Brentwood Mail Processing and Distribution Center—District of Columbia, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:1129–33.
13. Centers for Disease Control and Prevention, American Society for Microbiology, Association of Public Health Laboratories. Basic diagnostic testing protocols for level A laboratories for the presumptive identification of *Bacillus anthracis*. Available from: URL: http://www.asmusa.org/pcsrc/ban.asm.la.cp.102401f.pdf . Accessed October 2001.
14. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 11th informational supplement M100-S11. Wayne, Pennsylvania: The Committee; 2001.
15. Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships with *Bacillus anthracis*. J Bacteriol 2000;182:2928–36.

16. Centers for Disease Control and Prevention. Suspected cutaneous anthrax in a laboratory worker—Texas, 2002. MMWR Morb Mortal Wkly Rep 2002;51:279–81.
17. Hoffmaster AR, Fitzgerald CC, Ribot E, Mayer LW, Popovic T. Molecular subtyping of *Bacillus anthracis* and the 2001 bioterrorism-associated anthrax outbreak, United States. Emerg Infect Dis 2002;8;1111–6.
18. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
19. Mohammed MJ, Marston CK, Popovic T, Weyant RS, Tenover FC. Antimicrobial susceptibility testing of *Bacillus anthracis*: comparison of results obtained by using the National Committee for Clinical Laboratory Standards broth microdilution reference and Etest agar gradient diffusion methods. J Clin Microbiol 2002;40:1902–7.
20. Federal Bureau of Investigation. Photos of anthrax letters to NBC, Senator Daschle, and NY Post. Available from: URL: http://www.fbi.gov/pressrel/pressrel01/102301.htm . Accessed May 15, 2002.
21. Greene CM, Reefhuis J, Tan C, Fiore AE, Goldstein S, Beach MJ, et al. Epidemiologic investigations of bioterrorism-related anthrax, New Jersey, 2001. Emerg Infect Dis 2002;8;1048–55.
22. Tan CG, Sandhu HS, Crawford DC, Redd SC, Beach MJ, Buehler J, et al. Surveillance for anthrax cases associated with contaminated letters, New Jersey, Delaware, and Pennsylvania, 2001. Emerg Infect Dis 2002;8;1073–7.
23. Traeger MS, Wiersma ST, Rosenstein NE, Malecki JM, Shepard CW, Raghunathan PL, et al. First case of bioterrorism-related inhalational anthrax in the United States, Palm Beach County, Florida, 2001. Emerg Infect Dis 2002;8;1029–34.
24. Hsu VP, Lukacs SL, Handzel T, Hayslett J, Harper S, Hales T, et al. Opening a Bacillus anthracis-containing envelope, Capitol Hill, Washington, D.C.: the public health response. Emerg Infect Dis 2002;8;1039–43.
25. Dewan PK, Fry AM, Laserson K, Tierney BC, Quinn CP, Hayslett JA, et al. Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. Emerg Infect Dis 2002;8;1066–72.
26. Centers for Disease Control and Prevention. Interim guidelines for investigation of and response to *Bacillus anthracis* exposures. MMWR Morb Mortal Wkly Rep 2001;50:987–90.
27. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1008–10.
28. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and adverse events from antimicrobial prophylaxis. MMWR Morb Mortal Wkly Rep 2001;50:973–6.
29. Centers for Disease Control and Prevention. Additional options for preventive treatment for persons exposed to inhalational anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1142,1151.
30. Shepard CW, Soriano-Gabarro M, Zell ER, Hayslett J, Lukacs S, Goldstein S, et al. Antimicrobial postexposure prophylaxis for anthrax: adverse events and adherence. Emerg Infect Dis 2002;8;1124–32.
31. Jefferds MD, Laserson K, Fry AM, Roy S, Hayslett J, Grummer-Strawn L, et al. Adherence to antimicrobial inhalational anthrax prophylaxis among postal workers, Washington, D.C., 2001. Emerg Infect Dis 2002;8;1138–44.
32. Williams JL, Noviello SS, Griffith KS, Wurtzel H, Hamborsky J, Perz JF, et al. Anthrax postexposure prophylaxis in postal workers, Connecticut, 2001. Emerg Infect Dis 2002;8;1133–7.
33. Brachman P, Friedlander A. Inhalation anthrax. Ann N Y Acad Sci 1980;353:83–93.
34. Brookmeyer R, Blades N. Prevention of inhalational anthrax in the U.S. outbreak. Science 2002;295:1861.
35. Broad WJ, Johnston D. Anthrax sent through mail gained potency by the letter. The New York Times 2002 May 7.
36. Collingwood J. F.B.I.'s anthrax inquiry. The New York Times 2002 May 18.
37. Ashford DA, Rotz LD, Perkins BA. Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practice (ACIP). MMWR Morb Mortal Wkly Rep 2000;49(No. RR-15).
38. Centers for Disease Control and Prevention. Bioterrorism alleging use of anthrax and interim guidelines for management—United States, 1998. MMWR Morb Mortal Wkly Rep 1999;48:69–74.
39. Brachman PS, Plotkin SA, Bumford FH, Atchison MA. An epidemic of inhalation anthrax. II. Epidemiologic investigation. Am J Hyg 1960;72:6–23.
40. Barakat LA, Quentzel HL, Jernigan JA, Kirschke DL, Griffith K, Spear SM, et al. Fatal inhalational anthrax in a 94-year-old Connecticut woman. JAMA 2002;287:863–8.
41. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax—Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1077–9.
42. Webb GF, Blaser MJ. Mailborne transmission of anthrax: modeling and implications. Proc Natl Acad Sci USA 2002;99:7027-32.
43. Gerberding JL, Hughes JM, Koplan JP. Bioterrorism preparedness and response: clinicians and public health agencies as essential partners. JAMA 2002;287:898–900.
44. Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Anthrax as a biological weapon, 2002: updated recommendations for management. JAMA 2002;287:2236–52.

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First Case of Bioterrorism-Related Inhalational Anthrax in the United States, Palm Beach County, Florida, 2001

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On October 4, 2001, we confirmed the first bioterrorism-related anthrax case identified in the United States in a resident of Palm Beach County, Florida. Epidemiologic investigation indicated that exposure occurred at the workplace through intentionally contaminated mail. One additional case of inhalational anthrax was identified from the index patient's workplace. Among 1,076 nasal cultures performed to assess exposure, *Bacillus anthracis* was isolated from a co-worker later confirmed as being infected, as well as from an asymptomatic mail-handler in the same workplace. Environmental cultures for *B. anthracis* showed contamination at the workplace and six county postal facilities. Environmental and nasal swab cultures were useful epidemiologic tools that helped direct the investigation towards the infection source and transmission vehicle. We identified 1,114 persons at risk and offered antimicrobial prophylaxis.

In Florida, human anthrax has been rare; among eight human cases reported in Florida in the 20th century, the most recent was a cutaneous case in 1974 (1). On October 2, 2001, a 63-year-old Florida man was hospitalized for a nonlocalizing severe illness that began 2 days earlier, characterized by fever, chills, sweats, fatigue, and malaise, which progressed to vomiting, confusion, and incoherent speech. No history of cough, dyspnea, abdominal pain, diarrhea, or skin lesions was reported. On October 4, the Florida Department of Health (FDOH) Bureau of Laboratories confirmed B. anthracis from a culture of cerebrospinal fluid. The patient's condition deteriorated, and he died 3 days after admission (2).

After anthrax was confirmed and in consideration of possible bioterrorism, we initiated an investigation to determine the extent and source of the event, develop control strategies, and protect potentially exposed persons. This report summarizes the findings of our epidemiologic investigation.

Methods

Case Investigation

We performed a detailed investigation of the index patient's exposures during the 60 days before his illness. We visually inspected and obtained culture specimens for *Bacillus anthracis* at locations he visited during the 60-day period, including his home, recreational destinations, retail outlets patronized, and workplace. Initial samples from the workplace were from the patient's work area and the company mailroom and photo library, as well as air ventilation filters.

Case-Finding and Surveillance

A confirmed case of anthrax was defined as a clinically compatible cutaneous, inhalational, or gastrointestinal illness confirmed as anthrax by laboratory tests, including 1) isolation of B. anthracis from an affected tissue or site or 2) other laboratory evidence of B. anthracis infection based on at least two supportive laboratory tests (3). Supportive laboratory tests included polymerase chain reaction (PCR) (4) of DNA from

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patient fluid from a normally sterile site, immunohistochemical staining of patient tissue samples, and enzyme-linked immunosorbent assay serologic tests to detect immunoglobulin G (IgG) response to *B. anthracis* protective antigen (PA) (5).

We implemented case-finding through daily chart review in Palm Beach County intensive-care units (ICUs) and regionally in ICUs in North Carolina, where the index patient had traveled during the potential exposure period. ICU patients who had blood or cerebrospinal fluid cultures performed within 24 hours of hospital admission had more detailed chart reviews and interviews. If anthrax was not ruled out, further interviews were done with patients, family members, and medical providers. Laboratory testing for B. anthracis and other potentially causative pathogens was offered if indicated. Nearby counties implemented similar case-finding efforts in ICUs and emergency departments.

We initiated enhanced surveillance locally through alerts to medical examiners and statewide through requests to laboratory directors to forward to the FDOH laboratories any cultures suspicious for *Bacillus* species isolated from sterile sites. A statewide veterinary alert was issued for cases of anthrax in animals. All case-finding surveillance was retrospective to September 11, 2001, and prospective beginning October 5.

Surveillance in Potentially Exposed Groups

Workplace-exposed persons were defined as those who, within 60 days of onset of illness in the index patient, spent >1 h in the building where he worked. On October 3 through the employer, on October 8 through press releases and media briefings, and on October 8–10,13, 17, and 19 through information bulletins, we asked workplace-exposed persons and medical personnel caring for them to report influenzalike illness or skin lesions to the FDOH. Beginning October 8, hospitals were notified through infection-control professionals and public health alerts.

We obtained nasal swabs from workplace-exposed persons while dispensing prophylactic antibiotics on October 8–10 and from workers who handled trash at the workplace on October 13. Immediately after specimens were obtained, nasal swabs were applied to sheep-blood agar culture medium plates and transported to the Florida Public Health Laboratory. *B. anthracis* was confirmed in nonmotile, nonhemolytic isolates by gamma-phage lysis and PCR and later by detection of *B. anthracis* capsule and cell-wall antigens with direct fluorescent antibody tests. Testing for serum IgG antibody response to the PA component of the anthrax toxins was offered on October 10, 13, 17, and 19 to workplace-exposed persons.

We conducted interviews to investigate contaminated mail as an anthrax transmission vehicle at the workplace and to estimate incubation periods among anthrax patients. Persons who reported seeing or handling mail perceived as unusual or suspicious and persons with suspected anthrax exposure based on nasal swab cultures or preliminary serologic test results were interviewed to describe details surrounding unusual mail incidents as well as their routine exposure to the mail.

On October 12, we obtained nasal swabs from postal workers most likely to have handled contaminated mail at two county postal facilities that supplied mail to the workplace. We initiated anthrax surveillance on October 25 among postal employees in Palm Beach County through postal worker illness reports, a toll-free hotline for postal employees, and hospital infection-control professional reports of postal worker hospitalizations in Palm Beach County.

Environmental Investigation

We collected bulk objects (e.g., filters from heating, ventilation, and air conditioning [HVAC] units, mail, soil samples) and swab, wipe, vacuum, and air specimens to test for *B. anthracis* environmental contamination by standard collection and shipping techniques (6). Control samples were routinely performed.

After contamination was confirmed at the workplace, we performed focused environmental sampling on October 8–10. Samples were obtained at work areas of the index patient and persons identified with potential *B. anthracis* exposures through nasal swab cultures, preliminary serologic test results, and interviews. Samples were also obtained from trash receptacles, items removed from the building, and the company mail van. Subsequent sampling throughout the 68,000 square-foot, three-story building was performed on October 25–November 8, 2001, to characterize the extent of contamination in the workplace. Samples were obtained from all floors, the parking garage, and the roof.

Beginning on October 12, 2001, we obtained surface samples for cultures at Palm Beach County postal facilities that processed workplace mail. We obtained samples from mail facilities sequentially, in reverse order of a route the mail most likely followed to arrive at the workplace. Facilities from postal routes serving two workplace buildings were tested. One route included three postal facilities that process >99% of mail the workplace received, and another route included four other postal facilities that might process workplace mail if the mail had been sent to a previous office, vacated by the company 13 months earlier. We sampled areas in each facility where workplace mail was most likely to have been processed.

Selected environmental and clinical specimen isolates of *B. anthracis* were analyzed by determining base-pair sequences in designated portions of isolate DNA to characterize subtypes, and sequences were then compared. Base sequence analysis was performed by multiple-locus variable-number of tandem repeat typing analysis (MLVA) techniques (7).

Prophylaxis and Control Measures

We offered prophylactic antibiotics for *B. anthracis* to workplace-exposed persons (8). Until the risk for Florida postal workers could be assessed, we initiated prophylaxis for selected postal workers most likely to have handled workplace mail at two local postal facilities. Subsequent adjunct vaccination was later made available for workplace-exposed persons (9).

Results

Case Investigation

An autopsy of the index patient supported the diagnosis of inhalational anthrax. Autopsy findings included markedly enlarged hemorrhagic mediastinal lymph nodes on gross examination and laboratory detection of *B. anthracis* by immunohistochemical tests in mediastinal lymph nodes, spleen, liver sinusoids, and phagocytic cells.

The patient had no reported exposure typically associated with naturally occurring anthrax, including exposure to animals or animal products potentially harboring *B. anthracis* spores. He worked as a photo editor for a national media company that produces tabloid newspapers and other publications. He bicycled and fished for recreation, and his only travel in the 60 days before symptom onset on September 30 was a 5-day automobile trip to North Carolina. No typical naturally occurring anthrax sources were seen at any location inspected, and no *B. anthracis* contamination was detected among 44 samples from nonworkplace specimens. *B. anthracis* was identified in 2 of 12 specimens obtained on October 5: from the index patient's computer keyboard and his mailbox in the company mailroom.

Workplace interviews regarding mail exposure showed that the index patient rarely handled or opened workplace mail, but co-workers recalled that he had examined a piece of stationery containing a fine, white, talc-like powder on September 19. The patient was observed holding the stationery close to his face as he looked at it over his computer keyboard.

Case-Finding and Surveillance

No anthrax cases were detected in Palm Beach County ICU patients, although six patients underwent extensive follow-up from >500 medical charts reviewed through October 31, 2001. No anthrax cases were reported through surveillance by medical examiners. An autopsy was performed to rule out anthrax in one case reported through surveillance of medical examiners and Palm Beach County ICUs, and the patient was determined not to have anthrax. Through 2001, FDOH laboratories reported no *B. anthracis* isolations among 293 clinical isolates received to rule out anthrax. No reports of veterinary anthrax were received through the Florida Department of Agriculture and Consumer Services. No anthrax cases were reported through nearby county case-finding efforts among persons not exposed in the workplace.

Surveillance among Potentially Exposed Groups

Among six workplace-exposed persons who were extensively evaluated after medical providers reported their illness, one was also identified among Palm Beach ICU patients, and inhalational anthrax was confirmed in another, a Miami-Dade County resident. This second case-patient was a 73-year-old mail distributor and co-worker of the index patient, who was reported by his medical provider on October 4. His illness began on September 28, and he was admitted to a hospital in Miami-Dade County on October 1, 2001. A nasal swab culture obtained on October 5 showed *B. anthracis*, but cultures from blood, bronchial washings, and pleural fluid, obtained after initiation of antibiotics, were negative. Two specimens of pleural fluid obtained on October 5 and 12 were tested by PCR and were positive for *B. anthracis*. Immunohistochemical staining of *B. anthracis* capsule and cell-wall antigens from pleural fluid cytology preparations and from transbronchial and pleural biopsy tissues obtained on October 5 and 12 were positive. Serial serum samples, obtained on October 7, 10, 11, and 17, indicated a serum IgG antibody response to the PA component of the anthrax toxin consistent with acute *B. anthracis* infection. The patient was treated with antibiotics and was discharged from the hospital on October 17 (10).

Of 1,076 nasal cultures obtained from workplace-exposed persons, two yielded *B. anthracis*. The first was the second case-patient, and the second was from an asymptomatic mail sorter in the same workplace. Nasal swab cultures obtained from two workers that handled workplace trash did not yield *B. anthracis*.

Interviews with employees regarding suspicious mail showed that the two workplace-exposed persons with nasal cultures positive for *B. anthracis* had extensive mail exposure. One, the second case-patient, was the workplace mail distributor; he did not generally open mail and did not recall handling or seeing any mail containing powder or described as unusual or as hate mail. He picked up 10,000–15,000 pieces of mail from the post office each weekday in the company mail van and distributed it at the workplace. The other co-worker, a 36-year-old woman, sorted mail and opened mail addressed to a periodical different from the one to which the index patient contributed. She recalled opening an envelope that released powder in her office on or about September 25. Afterwards, she discarded it in the trash without reading it. The letter most likely had arrived during the previous 2 weeks while she was on vacation. No other workplace mail likely to contain *B. anthracis* was suggested through further interviews.

Workplace information about exposure to suspicious mail indicated that the incubation period for both Florida case-patients was <12 days (Figure 1). The index patient had onset

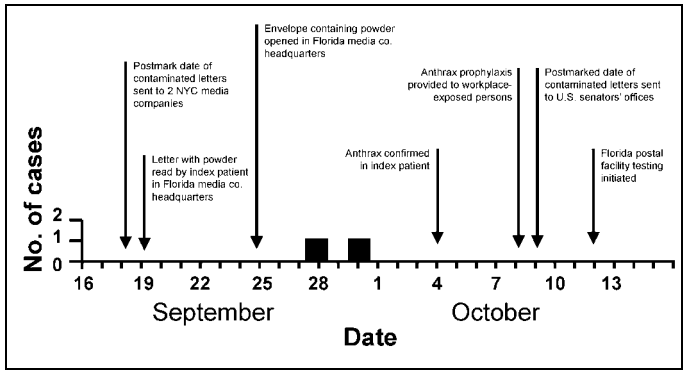

Figure 1. Dates of onset of symptoms of inhalational anthrax cases in Florida, and timeline of related events, September 16–October 16, 2001.

of illness 11 days after handling suspicious mail on September 19. The second case-patient had illness onset September 28, 9 days after the index-patient viewed suspicious mail on September 19 and 3 days after his co-worker opened a letter with powder in it on September 25.

Serial or paired serum tests for IgG antibody response to the PA component of the anthrax toxins were performed on serum of 436 workplace-exposed persons. No serum indicated a reaction consistent with acute *B. anthracis* infection except for that of the second case-patient. For most of the serologic tests, specimens were collected on October 10 and 17.

Among 32 postal workers who potentially handled workplace mail at two county postal facilities, 31 nasal cultures were obtained; none yielded *B. anthracis*. No anthrax cases were detected among 3,263 postal workers working at the 51 Palm Beach County postal facilities through the county postal worker surveillance system, which reported 226 illnesses and 7 hospitalizations during October 25–November 9, 2001.

Environmental Investigation

Of 136 investigation-directed environmental samples obtained during October 8–10 from the workplace and company mail van, 20 were positive, including 10 of 20 from the mailroom, 1 of 2 from the company mail van, 5 of 6 from the office of the asymptomatic mail-sorter who had a positive nasal culture and had opened a letter containing powder, 2 of 21 from the index patient's work area (at an incoming-mail desk near his workspace and a repeat sample from his computer keyboard), 1 of 9 in the text library, and 1 from the single basement ventilation filter sample. No *B. anthracis* contamination was detected from 8 trash receptacles or 2 roof ventilation filters, 28 bulk items removed from the building containing security camera information, 18 samples from a construction area, or 21 other samples from other work areas and the entrance lobby. Five samples from the third-floor HVAC ducts (three from the index patient's office and one from another office), and three samples from the first floor HVAC ducts (from the mailroom, an office where an envelope with powder was opened, and the text library) were negative.

Eighty-four of 460 workplace samples obtained during October 25–November 8 yielded *B. anthracis* (Figure 2). Isolates of *B. anthracis* were obtained from 66 of 247, 10 of 95, and 8 of 112 samples from the first, second, and third floors, respectively; none of 6 specimens were positive from the parking garage or roof vents. The northeast quadrant of the third floor, which contained executive office suites, a conference room, and storage areas, was the only quadrant of any floor without detected contamination. The index-patient's office was located on the third floor of the building. The mailroom (the work area of the second case-patient) and the office near the mailroom where a powder-containing letter was opened are both on the first floor.

No mail containing *B. anthracis* spores was recovered. Because workplace refuse is incinerated and waste receptacles did not show contamination, no environmental specimens were obtained from waste sites.

B. anthracis contamination was detected at six of seven postal facilities tested, from routes serving the current workplace headquarters and a former office. Contamination was not detected at a facility that receives mail addressed to the post office box of the former workplace office, last used 13 months earlier.

Molecular subtyping analysis (MLVA) was performed on one *B. anthracis* isolate recovered from a postal facility that processed workplace mail, 18 isolates recovered from the workplace, cerebrospinal fluid and blood culture isolates from the index patient, and two nasal swab isolates from workplace-exposed persons. All *B. anthracis* isolates tested were indistinguishable by MLVA.

Prophylaxis and Control Measures

Beginning October 8, we recommended 60-day antibiotic postexposure prophylaxis (2) to 1,114 workplace-exposed persons identified through employers and responses to public service announcements. We provided medication refills on October 17–19 and November 1 at a workplace branch office and as needed through the Palm Beach County Health Department. Beginning October 24, we attempted telephone contact

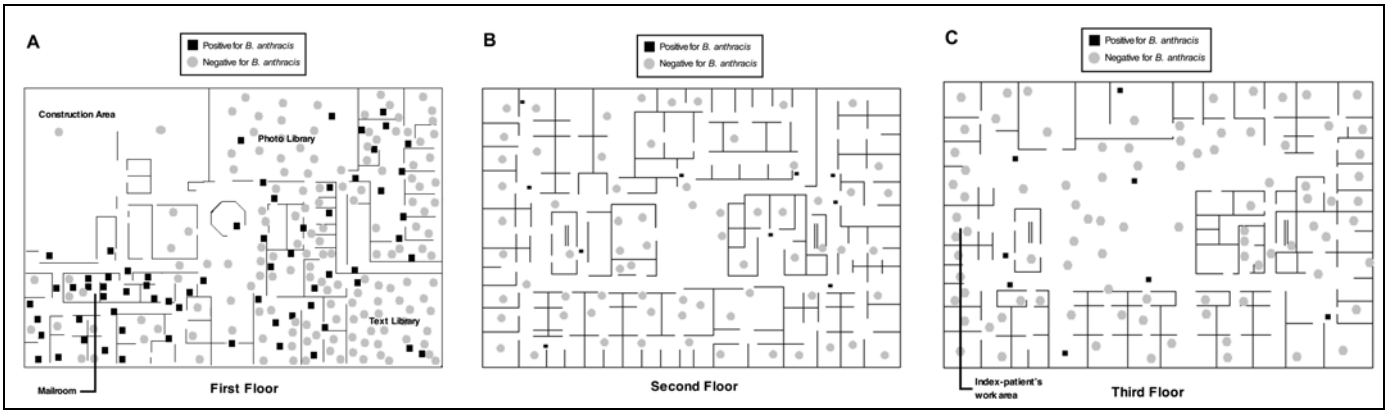

Figure 2. Environmental sample locations of specimens tested for *Bacillus anthracis* obtained October 25–November 8, 2001, on the three floors of the media company building where patients were employed, Palm Beach County, Florida. Sample locations of 59 negative specimens (including 46 air samples) are not depicted.

with persons who did not refill medications and advised them about our recommendations and how to obtain medications. Adjunct anthrax vaccination, available beginning December 22, was accepted by three workplace-exposed persons.

When the postal system risk assessment was initiated on October 12, antibiotic prophylaxis was offered to 32 postal workers who were most likely to have handled workplace mail at two local postal facilities. After we determined that at least 24 days had passed since contamination most likely took place in postal facilities, we did not recommend prophylactic antibiotics to Florida postal workers since more than two of the typical 1- to 7-day incubation periods for inhalational anthrax had passed, or two of the up to 12-day incubation periods estimated for the two Florida cases.

Discussion

This report describes the investigation of the first bioterrorism-related anthrax case identified in the United States. We detected two inhalational anthrax cases (including the index case) among workers of a Florida media company. Anthrax transmission and widespread environmental contamination throughout the workplace and in six local postal facilities most likely resulted from two letters containing *B. anthracis* spores delivered to the workplace.

The index patient's infection most likely occurred from inhalation of *B. anthracis* spores following a primary aerosolization, i.e., spores released into the air after opening a spore-containing letter. This scenario is consistent with coworkers' recollections that the index patient held a letter containing powder over his computer keyboard, as well as environmental samples showing contamination at his keyboard, an incoming-mail desk near his workspace, and his mailroom mailbox. The second case-patient did not recall opening or seeing a letter containing powder, and the mechanism of spore aerosolization resulting in his infection is unclear. He was likely exposed while delivering 10,000–15,000 mail pieces daily to the workplace mailroom; both the mailroom and mail van were contaminated with *B. anthracis* spores. He may have inhaled spores after mail was compressed or shaken during delivery or after he (unknowingly) or a co-worker opened a spore-containing envelope. A secondary aerosolization, i.e., spores resuspended in the air after settling to a surface following an initial release, may also have resulted in his infection.

Results from environmental specimens and nasal swab cultures helped guide the investigation and were especially useful when combined as epidemiologic tools. The first environmental sample yielding *B. anthracis*, from the index patient's work area, when paired with the first positive nasal swab culture, which was obtained from the second case-patient, indicated that the exposure source was at the workplace. Evidence that mail was the transmission vehicle was provided through two nasal swab cultures yielding *B. anthracis* from workplace mail handlers (one who recalled opening a letter containing powder) and results of environmental specimen cultures, revealing contamination in the workplace mail van and mail room. The usefulness of nasal swab cultures may have been limited by the interval of ≥13 days between the primary aerosolized spore exposures (letters opened on or about September 19 and 25) and the date nasal cultures were obtained (most on October 8). A high yield from nasal cultures would not be expected after ≥7 days had elapsed. One study showed that only one of eight nasal cultures from rhesus monkeys exposed to aerosolized *B. anthracis* spores yielded *B. anthracis* 7 days later (11). Environmental sampling was valuable independently in areas where no contamination was detected, by directing the investigation away from uncontaminated areas.

Environmental sampling revealed widespread contamination. However, the number or percentage of positive samples in a given area could not be used to quantify the contamination because quantitative spore counts were not performed when samples were cultured, a variety of sampling techniques were used (swabs, wipes, vacuum, and air sampling), and the distribution of samples obtained was not uniform.

This report documents the public health investigation into the first recognized case of anthrax due to intentional dissemination of *B. anthracis* spores in the United States. We demonstrated the usefulness of nasal swab cultures when combined with environmental specimen and epidemiologic data to identify the exposure site and vehicle used for anthrax transmission. Public health workers and clinicians should remain vigilant for anthrax because of the continued threat of bioterrorism.

Acknowledgments

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Dr. Traeger is a Public Health Service clinician and administrator in the Indian Health Service. During this investigation, he was working at the Florida Department of Health, Bureau of Epidemiology, for the Centers for Disease Control and Prevention as an Epidemic Intelligence Service officer.

References

1. Department of Health and Rehabilitative Services. State of Florida. Annual Report, 1974 (262). Tallahassee (FL): Florida Department of Health; 1974.
2. Bush L, Abrams B, Beall A, Johnson C. Index case of fatal inhalational anthrax due to bioterrorism in the United States. N Engl J Med 2001;345:1607–10.
3. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–3.

4. Hoffmaster AR, Meyer RF, Bowen M, Marston CK, Weyant RS, Barnett GA, et al. Evaluation and validation of a real-time polymerase chain reaction assay for rapid identification of Bacillus anthracis. Emerg Infect Dis 2002;8;1178-82.
5. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. Emerg Infect Dis 2002;8;1103-10.
6. Centers for Disease Control and Prevention. Comprehensive procedures for collecting surface environmental samples for culturing Bacillus anthracis. Available from: URL: http://www.bt.cdc.gov/Agent/Anthrax/environmental-sampling-apr2002.asp . Accessed August 19, 2002.
7. Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within Bacillus anthracis. J Bacteriol 2000;182:2928–36.
8. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1008–10.
9. Centers for Disease Control and Prevention. Additional options for preventive treatment for persons exposed to inhalational anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1142,1151.
10. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
11. Hail A, Rossi C, Ludwig G, Ivins B, Tammariello R, Henchal E. Comparison of noninvasive sampling sites for early detection of Bacillus anthracis spores from Rhesus monkeys after aerosol exposure. Mil Med 1999;164:833–7.

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First Case of Bioterrorism-Related Inhalational Anthrax, Florida, 2001: North Carolina Investigation

Jean-Marie Maillard,* Marc Fischer,† Kelly T. McKee, Jr.,* Lou F. Turner,* and J. Steven Cline*

The index case of inhalational anthrax in October 2001 was in a man who lived and worked in Florida. However, during the 3 days before illness onset, the patient had traveled through North Carolina, raising the possibility that exposure to *Bacillus anthracis* spores could have occurred there. The rapid response in North Carolina included surveillance among hospital intensive-care units, microbiology laboratories, medical examiners, and veterinarians, and site investigations at locations visited by the index patient to identify the naturally occurring or bioterrorism-related source of his exposure.

The index case of inhalational anthrax in October 2001 was in a man who lived and worked in Florida. However, during the 3 days before illness onset, he had traveled through North Carolina, raising the possibility that exposure to *Bacillus anthracis* spores could have occurred there. On October 4, concurrent investigations were initiated in Florida and North Carolina to identify the naturally occurring or bioterrorism-related source of his exposure. In less than a week, investigators isolated *B. anthracis* from the patient's place of employment in Florida (1,2). However, the history of travel to North Carolina had already resulted in a substantial public health effort in that state. We review the surveillance methods employed during the rapid response in North Carolina and discuss several lessons that may prove instructive for future investigations.

Methods

Surveillance Infrastructure

Retrospective syndrome- and laboratory-based surveillance for illnesses compatible with systemic anthrax infection was initiated on October 5 and continued for the 27 days from September 11 to October 6, 2001. Prospective surveillance was begun on October 7 and suspended on October 12. Based on the index patient's travel route, surveillance was undertaken in all 15 hospitals with intensive-care units (ICUs) in five North Carolina counties (combined population 1,258,980), and four regional referral centers in North Carolina (n=2) and South Carolina (n=2). These 19 hospitals have a total inpatient capacity of 5,720 beds.

A site coordinator, usually an infection control practitioner or hospital epidemiologist, was identified to lead the investigation at each hospital. The site coordinator communicated 1–2 times a day with a public health official designated as county anthrax surveillance officer. County surveillance officers reported cumulative data at daily conference calls with the state anthrax investigation team, which was based at the North Carolina Department of Health and Human Services (DHHS) in Raleigh. The state medical examiner, state veterinarian, and other experts (e.g., infectious disease clinicians) also participated in the daily conference calls to report any unexplained deaths identified in humans or farm animals and provide consultation as needed. Finally, a statewide information campaign was initiated by using electronic mailings to North Carolina health-care professionals and press releases to increase recognition by clinicians, raise public awareness, and provide contact information for any suspected cases.

Syndrome-Based Surveillance

For the 19 hospitals, investigators identified all patients admitted to the ICU from September 11 to October 7 who had blood or cerebrospinal fluid cultures obtained at the initial encounter. For patients meeting these criteria, the investigation team reviewed medical records to identify a subset of cases with one of four primary clinical syndromes, including fever and 1) severe respiratory disease (i.e., pneumonia or acute respiratory distress syndrome), 2) mediastinitis or mediastinal lymphadenitis, 3) meningitis, or 4) hemorrhagic gastroenteritis. Additional epidemiologic, clinical, and laboratory data were then obtained to define a specific cause of illness for patients with any of these syndromes.

Beginning October 7, hospital site coordinators reviewed emergency department, ICU, and autopsy logs daily to identify patients who died or were admitted with any of the four suspicious clinical syndromes. A standard report form was completed for each suspected case by abstracting the medical chart and, if needed, interviewing the patient's physician and family. Active suspected cases were maintained on a daily line list

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until a specific diagnosis or infectious agent had been identified or the possibility of anthrax had been excluded. A decision tree was developed to assist with finding and evaluating suspected cases (Figure).

Laboratory-Based Surveillance

Microbiology laboratory records from the 19 hospitals were reviewed both retrospectively (from September 11 to October 7) and prospectively (from October 7 to October 12) to identify suspicious bacterial isolates obtained from normally sterile sites (e.g., blood, cerebrospinal fluid, or pleural fluid). A suspicious isolate was defined as 1) nontyped *Bacillus* species, 2) unidentified nonhemolytic, nonmotile gram-positive rod, or 3) any other unidentified isolate that was discarded or sent to a referral laboratory. If the isolate was still available, additional phenotypic testing was performed at a local or reference laboratory to rule out *B. anthracis*. Concurrent with that process or if the isolate had been discarded, the patient's chart was abstracted to determine if the illness was compatible with systemic anthrax.

Site Investigation

Two teams of medical epidemiologists, industrial hygienists, and Federal Bureau of Investigation agents surveyed all North Carolina locations the patient visited before illness onset. The environmental investigation focused on two sites, including a relative's home and a rural tourist park. Family members who stayed or traveled with the index patient were interviewed. Recent illnesses and absences among the 90 employees at the park were reviewed. Available records (e.g., annual pass holders, credit card receipts) for approximately 700 persons who visited the park on the same day as the index patient were held for use in tracking patrons, if needed. Soil, water, vacuum filters, air filters, and swabs of selected surfaces were obtained from both locations to assess for *B. anthracis* spores. Samples were divided for testing at the North Carolina State Laboratory of Public Health and the Centers for Disease Control and Prevention.

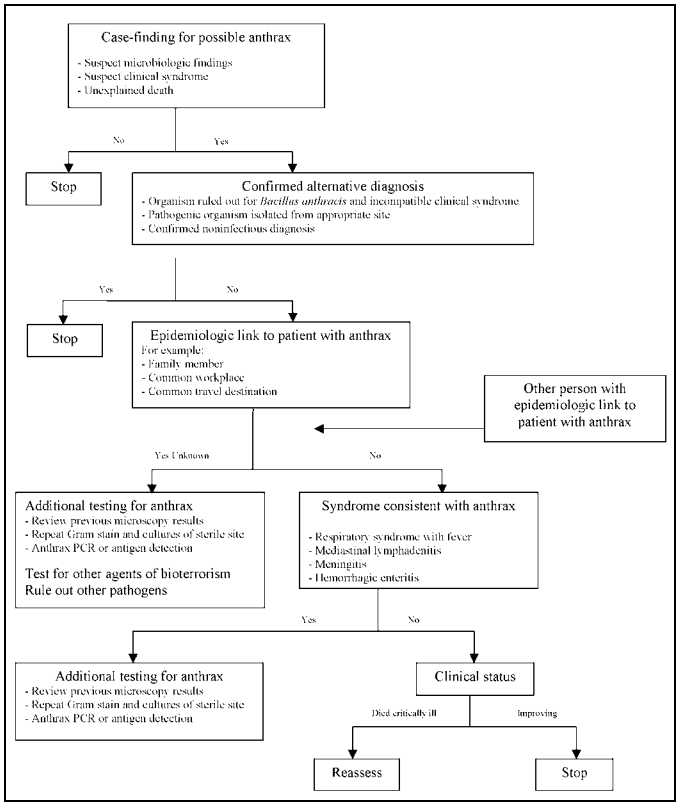

Figure. Decision analysis developed during the North Carolina investigation for identifying and evaluating patients with possible systemic anthrax. PCR, polymerase chain reaction.

Results

Syndrome-Based Surveillance

Investigators retrospectively identified 361 patients who were admitted to an ICU from September 11 to October 7 and had blood or cerebrospinal fluid cultures obtained at the initial encounter. Of these, 9 (2%) patients had a clinical syndrome of interest (all fever and severe respiratory disease) and required additional information to rule out a diagnosis of anthrax. The identification of suspected cases through retrospective case finding was completed by the end of the third day of the investigation. During October 7–12, prospective surveillance identified an additional five patients with fever and severe respiratory disease who died or were admitted to an ICU in one of the 19 hospitals under surveillance (Table).

Of the 14 cases of interest detected through hospital-based retrospective or prospective surveillance, 4 (29%) were fatal. None were due to anthrax. The state medical examiner identified one additional fatal case that warranted further evaluation in a county not included in the surveillance. This case of pneumonia and sepsis in a 10-year-old boy was subsequently attributed to a β-hemolytic streptococcus. No suspicious deaths of animals were reported to the state veterinarian during the relevant time period.

Laboratory-Based Surveillance

From September 11 to October 12, 10 isolates were identified through hospital microbiology laboratories that required additional investigation. All were either *Bacillus* species that had not undergone further identification or nonspecific gram-positive rods that had not been completely evaluated for hemolysis or motility. None of the patients from whom these bacteria were isolated had clinical courses consistent with inhalational anthrax, and none of the organisms were subsequently identified as *B. anthracis*.

Site Investigation

No relevant illnesses were identified in close contacts of the index patient in North Carolina or in other patrons or employees of the tourist park. No suspicious events (e.g., aerosol releases) or exposures were identified at any of the locations the patient visited. However, park employees noted that a cow had died of unknown causes in an adjacent orchard

Table. Surveillance methods used to identify potential cases of systemic anthrax or a source of exposure for the Florida index case of inhalational anthrax, North Carolina, October 2001

Type of surveillance	Targeted population or outcome	Locations under surveillance
Intensive-care unit	Patients with illness compatible with systemic anthrax infection[a]	19 hospitals in North and South Carolina[b]
Microbiology laboratory	Bacterial isolates potentially consistent with *Bacillus anthracis*[c]	19 hospitals in North and South Carolina
Medical examiner	Unexplained deaths possibly due to anthrax infection	Statewide
Veterinarian	Unexplained deaths in livestock	Statewide
Occupational	Unexplained illnesses or absences in employees	Tourist park visited by the index patient
Environmental	Evidence of *B. anthracis* spores	Residence of index patient's relative; tourist park visited by the index patient

[a]Clinical syndromes included fever and 1) severe respiratory disease, 2) mediastinitis or mediastinal lymphadenitis, 3) meningitis, or 4) hemorrhagic gastroenteritis.
[b]Based on the index patient's route of travel, surveillance occurred in all 15 hospitals with intensive-care units in five North Carolina counties, as well as four regional referral centers in North Carolina (n=2) and South Carolina (n=2).
[c]A suspicious isolate was defined as 1) nontyped *Bacillus* species, 2) unidentified nonhemolytic, nonmotile gram-positive rod, or 3) any other unidentified bacteria that was discarded or sent to a referral laboratory.

approximately 1 year earlier. Although the index patient had not visited this area, he had reportedly drunk water from a stream that traversed the tourist park after passing through the orchard. A total of 35 environmental samples were obtained from sites the index patient visited: 5 (14%) were from the relative's home and 30 (86%) from the tourist park, including soil from the area where the cow died and water from the stream. Cultures of all environmental specimens were negative for *B. anthracis*.

Discussion

In 1999, the North Carolina DHHS established short-term hospital-based surveillance in 18 counties to assess injuries and other medical consequences resulting from Hurricane Floyd. This experience was extremely useful in rapidly implementing syndromic surveillance during the anthrax investigation. Nevertheless, limited staffing, absence of electronic surveillance and reporting, the wide geographic area traversed by the patient, intense media scrutiny, and the simultaneous involvement of multiple public health and law enforcement agencies posed major challenges to the investigation.

The North Carolina anthrax investigation team required contributions from many persons of varied expertise, including epidemiologists, microbiologists, pathologists, veterinarians, infectious disease clinicians, infection control practitioners, engineers, industrial hygienists, health communicators, and law enforcement and emergency management personnel. The team operated under a command structure led by the North Carolina Department of Health and Human Services. Participating state and federal agencies were represented at both the investigation headquarters in Raleigh and on each field team. Conference calls that included all decision-making parties were held at the same time each day to rapidly disseminate information throughout team members, and set the specific priorities of the investigation for the next 24 hours. In addition, press releases were distributed regularly to minimize reporting inaccuracies, and dedicated spokespersons were identified to provide a clear and consistent message.

However, several factors could have helped the investigation run more efficiently. First, case definitions, surveillance methods, data collection forms, and informational materials had to be developed ad hoc throughout the investigation, resulting in delays in implementing surveillance, uncertainties as to the effectiveness of and person-hours required by the case-finding methods, and inefficiencies in the data collection process. Second, most of the communications and transfer of information during this investigation occurred by telephone and fax. Although this system was workable given its relatively small scale, it resulted in inefficient data management that would have been rapidly overwhelmed by additional cases or sites. Third, many persons and agencies involved in the investigation had not previously worked together, resulting in a lack of familiarity with their respective organization and capacity. Finally, substantial time and effort were needed during the investigation to educate health-care providers and public health practitioners about the epidemiology and clinical manifestations of inhalational anthrax.

This investigation and its ramifications provided an important learning opportunity and impetus to better prepare for future bioterrorist attacks. Standard protocols, data collection instruments, and informational documents that can be adapted to specific situations are being developed to minimize delays and avoid omissions. In North Carolina, resources are also being used to 1) establish state and regional teams trained in bioterrorism response and 2) develop a statewide Health Alert Network. North Carolina's network will be a secure multidirectional electronic network through which the state health department can rapidly communicate with hospitals, clinicians, and public health and law enforcement authorities. This new infrastructure will allow for an efficient flow of information during future investigations and provide surge capacity to better respond to requests for assistance at the local level. In addition, health professionals are being educated statewide to better recognize the clinical manifestations of biologic agents that may be used in terrorism. These efforts may build on lessons learned from the fall of 2001 to provide a more rapid,

comprehensive, and efficient response to public health emergencies.

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References

1. Centers for Disease Control and Prevention. Notice to readers: ongoing investigation of anthrax—Florida, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:877.
2. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–3.

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Opening a *Bacillus anthracis*–Containing Envelope, Capitol Hill, Washington, D.C.: The Public Health Response

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On October 15, 2001, a U.S. Senate staff member opened an envelope containing *Bacillus anthracis* spores. Chemoprophylaxis was promptly initiated and nasal swabs obtained for all persons in the immediate area. An epidemiologic investigation was conducted to define exposure areas and identify persons who should receive prolonged chemoprophylaxis, based on their exposure risk. Persons immediately exposed to *B. anthracis* spores were interviewed; records were reviewed to identify additional persons in this area. Persons with positive nasal swabs had repeat swabs and serial serologic evaluation to measure antibodies to *B. anthracis* protective antigen (anti-PA). A total of 625 persons were identified as requiring prolonged chemoprophylaxis; 28 had positive nasal swabs. Repeat nasal swabs were negative at 7 days; none had developed anti-PA antibodies by 42 days after exposure. Early nasal swab testing is a useful epidemiologic tool to assess risk of exposure to aerosolized *B. anthracis*. Early, wide chemoprophylaxis may have averted an outbreak of anthrax in this population.

In the fall of 2001, a series of envelopes containing *Bacillus anthracis* spores were sent via the U.S. Postal Service (USPS) to cities in Florida and New York. Consequently, many persons, including staff on Capitol Hill, received training on how to respond to suspicious envelopes that might contain *B. anthracis* spores. This training was based on previously prepared recommendations for a comprehensive response to biological attacks using *B. anthracis* (1–3). On October 15, 2001, an envelope addressed to Senator Tom Daschle containing *B. anthracis* spores was opened by one of his staff members. While the bioterrorism events in Florida and New York came to the attention of public health authorities only when persons were diagnosed (4–7) with anthrax, the event on Capitol Hill was different—the presence of *B. anthracis* spores was suspected immediately, allowing appropriate response and prompt initiation of chemoprophylaxis in exposed persons. A known source of exposure allowed a rapid epidemiologic investigation, using nasal swab cultures for *B. anthracis*, environmental sampling, and serologic testing. Although previous epidemiologic studies have used nasal swabs and serologic tests to assess *B. anthracis* exposure and subclinical (asymptomatic) infection in endemic and outbreak settings (8–11), the usefulness of these tools in the context of a bioterrorism event is not known.

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We describe here the initial public health response to the opening of the contaminated envelope on Capitol Hill and the epidemiologic methods used to determine the exposed area and the population at risk for developing anthrax. While the public health response later included the letter traceback through the entire postal system, including identification and prophylaxis of at-risk USPS employees (12), we limit our discussion to Capitol Hill. The results and epidemiologic importance of environmental sampling for *B. anthracis*, although briefly mentioned, will be the focus of a separate paper.

Timeline of Events

On October 15, 2001, at 9:45 a.m., a staff member on the 6th floor of the Hart Senate Office Building (HSOB) in the office of Senate Majority Leader Tom Daschle cut open a taped business envelope containing a letter and a powdery substance (Table 1). Upon noticing a burst of dust, she placed the letter on the floor and notified the U.S. Capitol Police. Within 5 minutes of being notified, officers were at the scene. The hazardous device unit of the Capitol Police arrived minutes later. The officers and emergency response personnel, referred to as first responders, arrived with respiratory personal protective equipment (PPE) on hand, but equipment was not put on until after arrival at the scene. These officers tested the powder for *B. anthracis* spores twice, using commercial rapid tests. Preliminary results obtained within 15 minutes suggested that the powder contained *B. anthracis*. Laboratories at the U.S. Army Medical Research Institute of Infectious Diseases

Table 1. Timeline of events within the Hart Senate Office Building, Washington, D.C., October 15, 2001[a]

Time of day	Event /response
9:45 a.m.	Staff person opens letter containing *Bacillus anthracis* spores.
9:55	First responders arrive at scene.
10:00	Hazardous device unit arrives at scene and performs initial tests for *B. anthracis.*
10:15	First rapid test is positive for *B. anthracis.*
10:30	Ventilation system turned off. Second rapid test is positive. OAP begins nasal swab testing and antibiotic chemoprophylaxis distribution.
10:40	6th floor staff moved to 9th floor; swabbing continues; staff later moved to 5th floor.
3:00 p.m.	Senators Daschle and Feingold's staff allowed to go home.

[a]OAP, Office of the Attending Physician.

(USAMRIID) in Fort Detrick, Maryland, later confirmed these preliminary results.

At approximately 10:30 a.m., the ventilation system was shut off. Medical staff from the Office of the Attending Physician (OAP), U.S. Capitol, began collecting nasal swabs for *B. anthracis* culture from staff members in Senator Daschle's office, from staff in an adjacent office belonging to Senator Russell Feingold, and from the first responders; in addition, an initial 3-day antimicrobial postexposure prophylaxis regimen consisting of ciprofloxacin, 500 mg twice a day, was given to these persons. Only the person who opened the contaminated envelope removed and changed her clothing and was decontaminated with soap and water. All others washed their hands with soap and water.

Next, first responders led employees from the two 6th-floor offices to the 9th floor of the building, where further samples were taken from nares and clothing. After testing, these employees were led back to Senator Daschle's 5th-floor office, where other staff members were detained. At approximately 3:00 p.m., the staff members were allowed to go home.

Employees in other offices continued working until the close of business. The southwest quadrant of the building was closed the morning of October 16, and a decision was made to close the entire HSOB that evening. During the next 3 days, OAP continued to collect nasal swabs for *B. anthracis* for all HSOB employees present on October 15 and for others on Capitol Hill who requested these tests. OAP also gave those tested an initial 3-day antimicrobial prophylaxis, pending final confirmation of the presence of *B. anthracis* spores and results of the epidemiologic investigation.

Methods

A team from the Centers for Disease Control and Prevention (CDC) arrived in Washington D.C., on the morning of October 16 to begin the epidemiologic investigation. To identify the group of persons who needed prolonged antimicrobial prophylaxis on the basis of likely exposure to *B. anthracis* spores, we sought to define an exposure area of higher risk.

To identify other facilities that may have been contaminated with *B. anthracis* spores, the contaminated envelope was traced back through the congressional mail distribution system before its arrival in Senator Daschle's office. To define the exposure area for HSOB, we obtained floor diagrams for the 5th and 6th floors and information about the ventilation system from the Office of the Architect of the Capitol, which maintains and operates the U.S. Capitol complex. Multiple environmental samples were taken from these facilities by a variety of techniques (13).

The population at risk of developing anthrax was defined as persons in the exposed area during or after the time the contaminated envelope was processed or opened. To identify each person who may have been within the exposure area, employee lists were obtained from staff managers for each affected facility; in HSOB, managers for individual senators' offices within the defined exposure area were contacted to obtain employee and visitor lists. We identified responders within HSOB, such as law enforcement and medical personnel, by contacting supervisors for a comprehensive list of those who were in the area. To identify other visitors or nonemployees, press conferences were used to relay the appropriate information.

Within 9 hours of the initial event, nasal swab specimens were collected for all persons in Senator Daschle's and Senator Feingold's offices and for all first responders. As mentioned earlier, further specimens were collected by OAP, for 4 days after the opening of the contaminated envelope, from employees of HSOB and others on Capitol Hill. Specimens were collected with Dacron fiber-tipped sterile swabs and sent for *B. anthracis* culture at the National Naval Medical Center in Bethesda, Maryland. Persons with initial positive nasal swabs for *B. anthracis* had repeat nasal swabs at 7 days postexposure and were administered a questionnaire about symptoms consistent with anthrax disease. In addition, serum specimens were obtained from these persons and tested at the CDC Meningitis and Special Pathogens Laboratory for the presence of immunoglobulin (Ig) G antibodies to *B. anthracis* protective antigen (anti-PA) at 7, 21, and 42 days postexposure.

In collaboration with OAP, efforts were made to ensure that all exposed persons were contacted and that they received appropriate prophylaxis with ciprofloxacin, or in the cases of persons unable to tolerate a quinolone, with doxycyline. OAP closely monitored persons who came to the clinic with respiratory symptoms; follow-up surveys were later conducted on persons receiving long-term antibiotic prophylaxis.

Results

Defining the Exposure Area and Population at Risk

Within Capitol Hill, the traceback of the contaminated envelope before its arrival in Senator Daschle's office showed that it had been screened through a mail facility on P Street and then through the Senate nonpublic mailroom, located in the Dirksen Senate Office Building (Table 2). Nasal swabs for

B. anthracis in employees of both mail facilities were negative; however, since exposure to *B. anthracis* spores may have occurred during mail handling of the contaminated letter, the Dirksen mailroom and the entire P Street facility, which was an open warehouse, were defined as exposed areas. Additionally, positive environmental samples for *B. anthracis* were found in the mailroom in the Ford House Office Building, where mail to the House of Representatives is processed. Although the contaminated envelope did not pass through the Ford Building mailroom, the potential of aerosolization of spores from processing equipment, as well as the possibility of an additional contaminated envelope, warranted its designation as an exposed area.

Senator Daschle's suite is located on the 5th and 6th floors of the southeast quadrant, with an open internal staircase joining the floors. An adjacent suite occupied by staff of Senator Feingold has a similar layout. Both adjacent offices share a common hallway that serves as the main entry to the 6th-floor office, but no door connects the Daschle and Feingold suites. A single ventilation system supplies and exhausts air for the nine floors in the southeast quadrant, independently of other areas in the building.

In HSOB, where the primary release of *B. anthracis* spores occurred, all persons with nasal cultures positive for *B. anthracis* were clustered in and around Senator Daschle's office and were located on either the 5th or 6th floor (see below). Preliminary environmental sampling results were positive for *B. anthracis* spores from within the same rooms occupied by persons with positive nasal cultures. The location of the contaminated office was within the shared ventilation space of the southeast quadrant of the building. The exposure area in HSOB was thus defined as the southeast quadrant of the 5th and 6th floors. Within these four designated exposure areas (5th- and 6th-floor southeast quadrant, P Street facility, and the Dirksen and Ford Building mailrooms), 625 persons were identified as employees, visitors, or otherwise being within the exposed areas (Table 2). More than 2,000 persons received an initial 3-day course of antibiotics, but only the 625 persons from the defined exposure areas were recommended to receive 60 days of chemoprophylaxis.

Nasal Swabs Results

OAP obtained nasal swabs for *B. anthracis* culture from 2,172 persons during October 15–October 18, including the 625 persons identified at risk. Of these, 71 were known to be in the immediate exposure area within the first hour of the event in which the contaminated envelope was opened (Table 3); 65 were Senate staff, and 6 were first responders. A total of 28 persons had positive nasal cultures for *B. anthracis*; all positive results were from specimens obtained on October 15 between 10:30 a.m. and 7:00 p.m. The median age of these persons was 27 years (range 21–57). All persons positive for *B. anthracis* entered either Senator Daschle's or Senator Feingold's suites, with the exception of one responder who was in the hallway adjacent to Senator Daschle's office on the 6th floor but did not enter either suite. All 18 persons (including 5 first responders) in Senator Daschle's 6th-floor suite had positive nasal cultures; a much lower proportion had positive nasal swabs on the 5th-floor Daschle suite (28%) and 6th-floor Feingold suite (13%).

Table 2. Defined exposure areas and identification of persons at risk from *Bacillus anthracis*–containing envelope, Washington, D.C.

Defined exposure area	Environmental samples positive?	No. persons identified at risk	No. positive nasal swabs
SE quadrant, 5th and 6th floors, Hart Senate Office Building	Yes	442	28
P Street mail-processing facility	Yes	62	0
Mailroom, Dirksen Senate Office Building	Yes	40	0
Mailroom, Ford House Office Building	Yes	81	0
Totals		625	28

Repeat nasal swabs from the 28 persons with initially positive nasal cultures for *B. anthracis* were negative for all persons at 7 days postexposure. Serologic tests were negative for anti-PA IgG antibodies in all persons at 7, 21, and 42 days after exposure. To date, anthrax has not developed in anyone in this cohort or in the larger cohort of persons on Capitol Hill.

Discussion

Among the series of bioterrorism incidents during 2001 related to *B. anthracis*–contaminated envelopes, this event was unique because it was the first with a known source of exposure, enabling a rapid public health response by a multidisciplinary team including law enforcement officers, medical and public health personnel, laboratory personnel, industrial hygienists, and engineers. The known source enabled us to assess the usefulness of nasal swab cultures in determining exposure to *B. anthracis*.

The contaminated letter purportedly contained about 2 g of powder, with each gram reported to contain between 100 billion to 1 trillion spores (14). The recovery of *B. anthracis* from nasal cultures was limited to persons who were inside Senator Daschle or Feingold's offices or in the hallway joining the two offices. Nasal swab results suggest that the ventilation system

Table 3. Proportion of persons with positive nasal swabs for *Bacillus anthracis* in the immediate exposure area, by office and floor,[a] Capitol Hill, Washington, D.C.

Floor	Senate office	Persons in area	Positive nasal swabs (% positive)
6	Daschle	18	18 (100)
	Feingold	15	2 (13)
5	Daschle	25	7 (28)
	Feingold	12	0 (0)
Total		70	27 (39)

[a]One responder with a positive nasal swab who was in the 6th-floor hallway did not enter the Daschle or Feingold suites and was not included in this table.

played a very small role, if any, in the spread of anthrax spores in HSOB. Based on proportions of persons with positive nasal swabs, most dissemination likely occurred through room currents from the 6th to the 5th floor of the Daschle suite via an open staircase; closed doors that blocked air currents were most likely the reason a smaller proportion in Senator Feingold's office had positive nasal cultures despite being adjacent to Senator Daschle's office.

Swabs were taken within 1 day of the initial event from all 71 persons in the immediate exposure area, including those with positive nasal cultures for *B. anthracis*. However, in others with negative results, testing was not done for up to 4 days. Although these persons were located outside the immediate exposure area, it is uncertain whether prompt antibiotic administration, a delay in nasal swab testing, or both, may have had an effect on those nasal culture results. In one animal model involving macaques, large inhaled doses of anthrax spores in a controlled setting yielded *B. anthracis* in nasal swabs of all animals within 24 hours of exposure, and although sensitivity decreased as time progressed, positive nasal cultures were recovered in some macaques 1 week after exposure (15). In the Florida anthrax investigation, positive nasal cultures were detected in a person >1 week after presumed exposure (5). Repeat swabs from the persons with initially positive cultures were negative at 7 days postexposure, but prophylaxis administration may have influenced those results. The greatest sensitivity for recovery of *B. anthracis* can be achieved by obtaining nasal swab specimens as early as possible after recognized exposure.

Nasal swabs served as an epidemiologic tool; we considered the work locations of those with positive nasal swabs to be areas at risk for anthrax exposure. However, interpretation of positive or negative nasal swab results for individual risk assessment of anthrax disease has not been evaluated, and nasal swabs should not be used for this purpose. In the case of one person who died after exposure to anthrax, a nasal swab culture was negative (16). Likewise, environmental sampling may be a valuable component of assessment of areas of risk, but individual environmental samples are not sufficient to determine a person's risk for anthrax.

Two other issues deserve mention. First, the use of PPE may be an effective barrier to exposure to *B. anthracis* spores, although its efficacy could not be addressed in this investigation; no responder entering Senator Daschle's office wore PPE before entering the office, and all had positive nasal swabs. Second, while subclinical anthrax infection has been documented in persons with continuous exposure to *B. anthracis* spores (9), the lack of serologic conversion in persons with positive nasal cultures suggests that no apparent asymptomatic infection occurred during this event, when prophylaxis was promptly initiated and continued.

Since the initial events of October 15, more information has become available—four cases of inhalational anthrax, two of them fatal, occurred in USPS employees from the Washington, D.C., Postal Distribution Center where Senator Daschle's envelope was sorted (7,12,16), and a fifth case occurred in an employee of another mail facility, which receives government mail from the Washington, D.C., Distribution Center. These events led to new recommendations to expand the traceback for future events through the entire path to envelope origin. In addition, updated prophylaxis and treatment protocols, including options for vaccination, and subsequent recommendations for a comprehensive response to a bioterrorism attack involving *B. anthracis* have been published (17–21). In Table 4, specific recommendations are given for a comprehensive public health response and epidemiologic investigation that prevent further spread, identify and treat those at risk, and avoid mass administration of prolonged prophylaxis to persons not considered at risk for anthrax in the event of a future bioterrorist attack.

Table 4. Recommendations for public health response to, and epidemiologic assessment of, the opening of an envelope suspected of containing *Bacillus anthracis* spores

Proper training on handling suspicious envelopes and packages
Use of personal protective equipment
Rapid identification of *B. anthracis* spore
Shutdown of ventilation system
Evacuation of immediate and surrounding area
Prompt administration of antimicrobial prophylaxis, in conjunction with offering vaccine under appropriate circumstances, to persons in immediate area
Use of epidemiologic tools to define exposure area and assess risk in the surrounding area
Nasal cultures and environmental samples for *B. anthracis*
Floor diagrams
Building ventilation
Traceback of letter path from destination to origin

In conclusion, a rapid and coordinated public health response helped avert an anthrax outbreak by identifying and administering prophylaxis to persons at high risk for disease. Nasal swabs can provide useful information about the extent of exposure to *B. anthracis* spores to assist with defining groups at risk.

Epidemiologic assessment of risk for anthrax in persons in settings affected by a biological attack is complex, and much remains to be learned. In the meantime, a well-developed public health infrastructure, effective antimicrobial prophylaxis strategies, and effective guidelines for management based on past experiences are essential in our defense against future bioterrorism events.

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References

1. Centers for Disease Control and Prevention. Bioterrorism alleging use of anthrax and interim guidelines for management—United States, 1998. MMWR Morb Mortal Wkly Rep1999; 48:69–74.
2. Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, et al. Anthrax as a biological weapon. JAMA 1999;281:1735–45.
3. Centers for Disease Control and Prevention. Biological and chemical terrorism: Strategic plan for preparedness and response. MMWR Morb Mortal Wkly Rep 2000;49(RR-4).
4. Bush LM, Abrams BH, Beall A, Johnson CC. Index case of fatal inhalational anthrax due to bioterrorism in the United States. N Engl J Med 2001;345:1607–10.
5. Traeger MS, Wiersma ST, Rosenstein NE, Malecki JM, Shepard CW, Raghunathan PL, et al. First case of bioterrorism-related inhalational anthrax in the United States, Palm Beach County, Florida, 2001. Emerg Infect Dis 2002;8;1029–34.
6. Freedman A, Afonja O, Chang MW, Mostashari F, Blaser M, Perez-Perez G, et al. Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a 7-month-old infant. JAMA 2002;287:869–74.
7. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
8. Carr EA, Rew RR. Recovery of *Bacillus anthracis* from the nose and throat of apparently healthy workers. J Infect Dis 1957;100:169–71.
9. Norman PS, Ray GR, Brachman PS, Plotkin SA, Pagano JS. Serologic testing for anthrax antibodies in workers in a goat hair processing mill. Am J Hygiene 1960;71:32–7.
10. Sirisanthana T, Nelson KE, Ezzell JW, Abshire TG. Serological studies of patients with cutaneous and oral-oropharyngeal anthrax from northern Thailand. Am J Trop Med Hyg 1988;39:575–81.
11. Harrison LH, Ezzell JW, Abshire TG, Kidd S, Kaufmann AF. Evaluation of serologic tests for diagnosis of anthrax after an outbreak of cutaneous anthrax in Paraguay. J Infect Dis 1989;160:706–10.
12. Dewan PK, Fry AM, Laserson K, Tierney BC, Quinn CP, Hayslett JA, et al. Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. Emerg Infect Dis 2002;8;1066–72.
13. Centers for Disease Control and Prevention. Evaluation of *Bacillus anthracis* contamination inside the Brentwood mail processing and distribution center—District of Columbia, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:1129–33.
14. Kennedy H. Daschle letter bombshell billions of anthrax spores. New York Daily News. October 31, 2001:5. Available from: URL: http://www.nydailynews.com
15. Hail AS, Rossi CA, Ludwig GV, Ivins BE, Tammariello RF, Henchal EA. Comparison of noninvasive sampling sites for early detection of *Bacillus anthracis* spores from Rhesus monkeys after aerosol exposure. Mil Med 1999;164:833–7.
16. Borio L, Frank D, Mani V, Chiriboga C, Pollanen M, Ripple M, et al. Death due to bioterrorism-related inhalational anthrax. JAMA 2001;286:2554–9.
17. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October, 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
18. Centers for Disease Control and Prevention. Updated recommendations for antimicrobial prophylaxis among asymptomatic pregnant women after exposure to *Bacillus anthracis*. MMWR Morb Mortal Wkly Rep 2001;50:960.
19. Centers for Disease Control and Prevention. Update: Interim recommendations for antimicrobial prophylaxis for children and breastfeeding mothers and treatment of children with anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1014–6.
20. Centers for Disease Control and Prevention. Additional options for preventive treatment for persons exposed to inhalational anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1142, 1151.
21. Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Anthrax as a biological weapon, 2002. JAMA 2002;287:2236–52.

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Bacillus anthracis Aerosolization Associated with a Contaminated Mail Sorting Machine

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On October 12, 2001, two envelopes containing *Bacillus anthracis* spores passed through a sorting machine in a postal facility in Washington, D.C. When anthrax infection was identified in postal workers 9 days later, the facility was closed. To determine if exposure to airborne *B. anthracis* spores continued to occur, we performed air sampling around the contaminated sorter. One CFU of *B. anthracis* was isolated from 990 L of air sampled before the machine was activated. Six CFUs were isolated during machine activation and processing of clean dummy mail. These data indicate that an employee working near this machine might inhale approximately 30 *B. anthracis*-containing particles during an 8-h work shift. What risk this may have represented to postal workers is not known, but this estimate is approximately 20-fold less than a previous estimate of sub-5 µm *B. anthracis*-containing particles routinely inhaled by asymptomatic, unvaccinated workers in a goat-hair mill.

In the fall of 2001, 22 cases of anthrax were confirmed or suspected throughout the eastern United States as a result of bioterrorist release of *Bacillus anthracis* spores (1). Ten cases (seven inhalational and three cutaneous) occurred in workers at postal facilities in which envelopes contaminated with *B. anthracis* spores were processed by high-speed sorting machines. Two contaminated envelopes passed through a sorting machine at the United States Postal Service Processing and Distribution Center in Washington, D.C. (Brentwood mail facility), on the morning of October 12. The facility was closed on October 21 after anthrax infection was diagnosed; four employees were eventually confirmed as having inhalational anthrax (2). During the 9-day period while the facility continued to operate, >2,000 employees processed >60 million pieces of mail. In addition to the primary aerosol to which workers may have been exposed, they may have had continual reexposure to *B. anthracis* spores during this period.

At the time of the anthrax release in the fall of 2001, little was known about the re-aerosolization potential of *B. anthracis* spores after initial dispersion. Much of what was known came from studies conducted by the United States and Canadian military biological defense programs, using surrogate biological agents dispersed outdoors at very high concentrations (10^5–10^8 agent-containing particles/m^2). These studies showed that re-aerosolization can occur, but risk is considered to be low (3,4). No information was available to answer similar questions about re-aerosolization risk in an indoor occupational setting such as a postal facility.

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To address the question of continued risk for workers, we conducted an expanded safety evaluation of the partially remediated mail facility. A stamp on one of the two contaminated envelopes indicated that it had passed through Delivery Bar Code Sorter machine no. 17 at the Brentwood mail facility. This sorter, which had been idle for >2 weeks, had been cleaned with 0.5% hypochlorite solution before our testing. We evaluated the potential health risk to workers near that sorter by activating it and conducting surface and air sampling.

Methods

Surface Sampling

Two surface sampling techniques were used. Rodac plates (65-mm tryptic soy agar [TSA] plates; Becton-Dickinson, Franklin Lakes, NJ) were pressed onto the surface being sampled. Immediately adjacent to the Rodac sampling site, swab sampling was performed with sterile rayon-tipped swabs moistened with a 0.5-mL solution of phosphate-buffered saline + 0.05% Tween 20 (PBS Tween). An approximately 100-cm^2 area was swabbed with sequential vertical, horizontal, and diagonal strokes. The swabs were individually placed in sterile, dry 15-mL conical tubes. Sampling focused on areas in the machine (electrical components, beneath belts, etc.) that were unlikely to have been cleaned with the topical bleach application.

Air Sampling

The ventilation system in the mail facility was turned off when the facility closed, and the system remained off during testing. We were unable to simulate the "blow-down" procedure (used to clean the sorter) during testing because the air

compressors for the air hoses had lost power. Machine operators typically use high-pressure hoses several times a day to clean accumulated dust and debris between mail-sorting runs. Two banks of 10-slit samplers were placed on two postal trolleys (approximately 5 feet above the floor) and connected to a vacuum pump. The slit sampler intake ports were approximately 10 inches above the trolley. Each of the samplers was loaded with 150-mm TSA plates. Slit sampler set "A" (SSSA) was placed next to the operator's station (at a location and height where workers would spend most of their time), and slit sampler set "B" (SSSB) was placed at the opposite end of the sorter (Figure 1). To measure the temporal patterns of re-aerosolization, the samplers operated sequentially; the intake port of slit sampler no.1 was opened, allowed to run for either 1 min (SSSA) or 2 min (SSSB), and then closed. Then slit sampler no.2 in the set was activated, and so on, until all 10 slit samplers in the set had been sequentially activated. The rate of air flow through each of the slit samplers was 33 L/min.

SSSB was activated and ran for 20 min while the sorter was turned off. SSSA was then activated and ran for 10 min. The plates were removed from all slit samplers and new plates were loaded. The SSSA and SSSB design characteristics determined the duration of sampling.

Both sets of slit samplers were activated simultaneously while the sorter was inactive. Approximately 1 min later, the sorter was started, and clean dummy mail was processed. After several false starts, continuous operation was achieved in approximately 2 min. The operation of the sorter was interrupted several times by jammed envelopes and quickly restarted each time, until the machine was turned off 1 min before the end of the 20-min sampling period. Postal officials reported that false starts, jamming, and restarting are common during routine operation of the machine.

As with the previous sampling, SSSA ran for 10 min and SSSB ran for 20 min. The plates were removed from the slit samplers and sealed in plastic bags. The bagged plates were taken out of the facility and the exteriors of the bags were decontaminated with 0.5% hypochlorite solution.

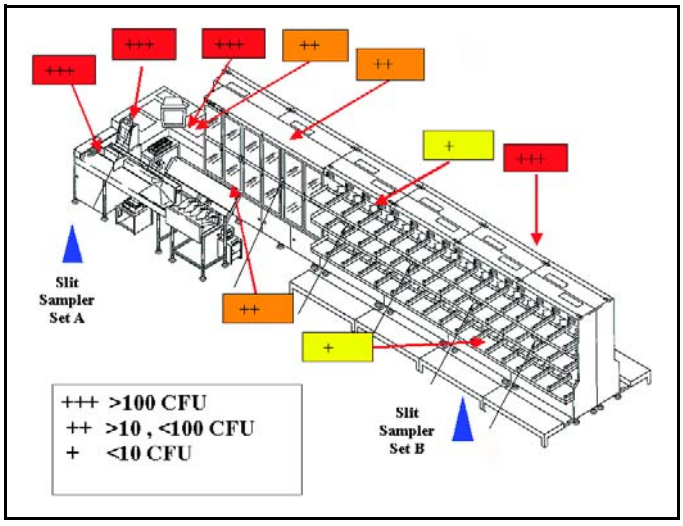

Figure 1. Sites and results of surface sampling for *Bacillus anthracis* with Rodac plates.

Mask Filters

The sampling team was outfitted with Canadian military C4 respirators with C7 canisters. The mask was equipped with 37-mm glass fiber collection filters mounted on the inlet port of the C7 canister, so that the entire inspirational volume of the investigators was sampled. The masked team members were located near the sorter to provide additional point sampling of respirable aerosol during the experiment. Team members were stationed at different work sites along the sorter and elsewhere in the facility to serve as point detectors. All mask filters were worn for at least 2 h.

Sample Handling and Processing

Environmental swabs and TSA plates from the Rodac plates and slit samplers were stored at 4°C until shipped and processed. All specimens were shipped at room temperature overnight to the Centers for Disease Control and Prevention. Swabs were placed in 1.5 mL PBS-Tween and vortexed for approximately 1 min. The solution was heat-shocked at 65°C for 30 min, and 100 µL was plated onto a sheep blood agar (SBA) plate. Rodac and slit sampler plates were incubated for approximately 12 h, and CFU were counted by visual inspection. All colonies suspected to be *Bacillus* spp. were subcultured on SBA plates. Identification and confirmatory testing of *B. anthracis* were done according to standard microbiologic procedures (5). The mask filters were removed in the facility and placed in sterile glass tubes. After transport, they were suspended in 3 mL of heart infusion broth and incubated at 35°C for 36 h, after which 10 µL of broth was plated onto SBA plates. The filters and remaining broth were heat-shocked at 65°C for 30 min, and 10 µL was plated onto SBA plates.

Statistical Analysis

Numbers of colonies detected in air sampling before and after the machine was activated were assessed with a one-sided one-sample test for difference in rates from a binomial distribution by using StatExact 4 v. 4.0.1. (Cytel Software Corp., Cambridge, MA).

Results

Surface Sampling

Surface sampling was done by two methods, Rodac plates and premoistened swabs, to establish that the machine was still contaminated with viable *B. anthracis* spores. Ten Rodac plate samples and 10 swab samples were taken on the sorter surfaces. Both the Rodac plates and swabs yielded growth of CFUs that were too numerous to count at 7 of the 10 sites. Two additional Rodac plates were positive with low levels of contamination (1 and 3 CFUs); these locations were negative by the swab method (Figure 1).

Air Sampling

A single colony of *B. anthracis* was identified on one of the SSSA plates (Figure 2) during 10 min of sampling before the sorter was activated. No *B. anthracis* was identified on any of the SSSB plates during the 20 min of sampling.

After the sorter was activated, SSSA and SSSB ran for 10 min and 20 min, respectively. A single colony of *B. anthracis* was identified during minute 10 of sampling by SSSA (Figure 2; Table). SSSB identified a single colony of *B. anthracis* at each of minutes 1, 5, 7, 8, and 19.

Five investigators wore mask filters while the sorting machine was inactive; four investigators changed to new mask filters while the sorting machine was active. All mask filters were negative by culture.

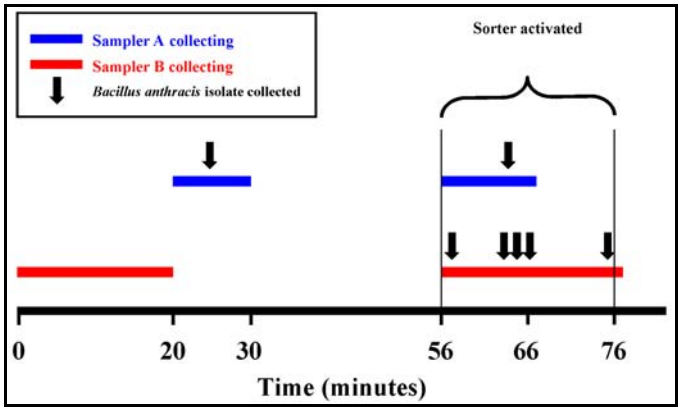

Figure 2. *Bacillus anthracis* air sampling: Slit Sampler Set A collected air samples for 10 min before and 10 min after the mail sorter was turned on. Slit Sampler Set B collected for 20 min during each period. Total air-flow rate, 33 L/min in both samplers.

Discussion

New questions have arisen as public health authorities have investigated and responded to the intentional release of *B. anthracis* in the United States. Studies by Canadian investigators with a sophisticated preparation of *Bacillus globigii* have shown that a contaminated envelope may, even unopened, cause a substantial primary aerosol event (6). In light of this new appreciation, we investigated whether, after a remote contamination event and initial decontamination, a Delivery Bar Code Sorter machine could be a continual source of aerosolized *B. anthracis* spores and, if so, whether the particle concentration in the air could be estimated.

Initial reports indicated that no specific remediation had yet been undertaken on the contaminated machine. Subsequently, we learned that the surface of the sorter implicated in processing the contaminated envelopes had been cleaned with 0.5% hypochlorite solution. We proceeded with testing because the expectation was that topical cleaning would provide only fractional decontamination of a contaminated machine. By focused sampling, we found that, despite topical cleaning, the sorter remained contaminated with *B. anthracis*. By either swab technique or Rodac plates, 9 of 10 sites on the machine were positive and 4 sites produced *B. anthracis* colonies that were too numerous to count.

Air sampling detected *B. anthracis* before and after the sorter was activated. Before the sorter was turned on, the samplers detected a single *B. anthracis*-containing particle (0.0010 agent-containing particles per liter of air [ACPL]). Six colonies of *B. anthracis* (0.0061 ACPL) were identified in the 990 L of air sampled after sorter activation. The difference between the number of *B. anthracis*-containing particles detected by the samples collected as background and those collected after the sorter was activated was not significant at the 0.05 level (p=0.06); however, analysis suggests a trend toward a significant increase.

Environmental surface sampling done shortly after the Brentwood mail facility was closed found widespread contamination of the facility with *B. anthracis* (7). Both aerosolization of *B. anthracis* spores and direct cross-contamination of surfaces were considered likely mechanisms for contamination. Approximately 30 h of air sampling with open-faced 37-mm mixed cellulose ester filters (0.8-µm pore size) was negative. The previous report of negative air sampling despite extensive testing suggests our detection of airborne *B. anthracis* while the sorter was inactive may have been spurious and possibly related to investigator activities while the experiment was being set up.

Based on these concentrations and assuming 100% sampler collection efficiency, the estimated number of *B. anthracis*–containing particles that a worker might inhale near this activated sorter can be calculated. If we assume a normal ventilation rate (10 L/min), during 8 h working around this partially cleaned, but still contaminated sorter, a worker might be expected to inhale approximately 30 *B. anthracis*-containing particles. This finding of very low-level airborne *B. anthracis* contamination is supported by the negative testing of the mask filters. If all the airborne particles are assumed to be of optimal size for inhalation, this estimate is approximately 100-fold less than the lower boundary of the 50% lethal dose estimates for inhalational anthrax in nonhuman primate studies (8). This number is also approximately 20-fold less than estimates of the number of routinely inhaled *B. anthracis*–containing particles from a 1960 study of asymptomatic, unvaccinated workers in a goat-hair mill in Pennsylvania (9). In that study, investigators calculated that, in an 8-h workday, workers

Table. Results of air sampling after Delivery Bar Code Sorter machine no. 17 was activated

Time (min)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Sampler A										+[a]										
Sampler B	+				+		+	+											+	

[a]+, positive for *Bacillus anthracis* CFU.

inhaled >1,300 viable *B. anthracis*-containing particles, 510 of which were <5 :m in size. Thus, although detected in the Brentwood facility, airborne contamination was at a relatively low level.

The comparison of this type of exposure with nonhuman primate anthrax data and historical industrial anthrax data is problematic for several reasons. Our understanding of human infection risk at very low-dose *B. anthracis* exposures is limited, as illustrated by the death from inhalational anthrax of an elderly Connecticut woman for whom no exposure could be determined, despite extensive environmental testing of her home and areas she frequented (1). A well-known contributor to the rate of alveolar deposition of a bioaerosol is the particle size distribution; because the slit sampling method does not measure the aerodynamic particle size distribution, we were unable to measure this attribute. Finally, historical comparisons to goat-hair mill workers are limited by the unknown contributions of prior host immunity, incomplete surveillance, and the lack of additional environmental sampling data other than the study from Pennsylvania.

This study shows that a mail sorter may remain contaminated, as indicated by surface sampling, many days after processing *B. anthracis*–contaminated letters and despite topical bleach cleaning. In addition, even after processing >1.2 million subsequent letters, as this sorter did, aerosolized *B. anthracis*–containing particles can still be detected around a contaminated sorter when active, at a level likely increased over background levels. At the time of our study, the level of *B. anthracis*–containing particles around this contaminated sorter at Brentwood was low, but any level of aerosolized *B. anthracis* spores is undesirable in this occupational setting.

Further studies are essential to define the risks of inhalational anthrax in the settings of both primary and secondary aerosolization of *B. anthracis* spores. In anticipation of potential future *B. anthracis* exposures, re-aerosolization potential should be evaluated in other environments, such as an office setting. In addition, size stratification of the re-aerosolized portion of a primary release should be part of any testing, to give some guidance as to risk stratification for exposed persons. Finally, better understanding of human health risk of low-dose exposure of *B. anthracis* spores is critical to guide optimal public health response.

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

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References

1. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax—Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1077–9.
2. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–18.
3. Chinn KSK, Adams DJ, Carlon HR. Hazard assessment for suspension of agent-contaminated soil. Joint Operational Test & Information Directorate, U.S. Army Dugway Proving Ground. 1990. Publication DPG/JOD-91/002.
4. Resnick IG, Martin DD, Larsen LD. Evaluation of the need for detection of surface biological agent contamination. Life Sciences Division, U.S. Army Dugway Proving Ground. 1990. Publication DPG-FR-90-711.
5. Logan NA, Turnbull PCB. Bacillus and recently derived genera. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of clinical microbiology, vol. 7. Washington: American Society for Microbiology Press; 1999; p. 357–69
6. Kournikakis B, Armour SJ, Boulet CA, Spence M, Barsons B. Risk assessment of anthrax threat letters, Suffield: DRES Technical Report TR 2001-048; 2001 September.
7. Centers for Disease Control and Prevention. Evaluation of *Bacillus anthracis* contamination inside the Brentwood Processing and Distribution Center—District of Columbia, October, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1129–32.
8. Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, et al. Anthrax as a biological weapon: medical and public health management. JAMA 1999;281:1735–45.
9. Dahlgren CM, Buchanan LM, Decker HM, Freed SW, Phillips CR, Brachman PS. *Bacillus anthracis* aerosols in goat hair processing mills. Am J Hyg 1960;72:24–31.

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Epidemiologic Investigations of Bioterrorism-Related Anthrax, New Jersey, 2001

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At least four *Bacillus anthracis*–containing envelopes destined for New York City and Washington, D.C. were processed at the Trenton Processing and Distribution Center (PDC) on September 18 and October 9, 2001. When cutaneous anthrax was confirmed in a Trenton postal worker, the PDC was closed. Four cutaneous and two inhalational anthrax cases were identified. Five patients were hospitalized; none died. Four were PDC employees; the others handled or received mail processed there. Onset dates occurred in two clusters following envelope processing at the PDC. The attack rate among the 170 employees present when the *B. anthracis*–containing letters were sorted on October 9 was 1.2%. Of 137 PDC environmental samples, 57 (42%) were positive. Five (10%) of 50 local post offices each yielded one positive sample. Cutaneous or inhalational anthrax developed in four postal employees at a facility where *B. anthracis*–containing letters were processed. Cross-contaminated mail or equipment was the likely source of infection in two other case-patients with cutaneous anthrax.

On October 4, 2001, inhalational anthrax was diagnosed in a Florida man who had no known exposure risk factors (1). The following week, cases of cutaneous anthrax in persons exposed to letters containing a suspicious powder were reported in New York City. The initial investigation showed that four envelopes containing *Bacillus anthracis* spores were mailed through the U. S. Postal Service (USPS) to media outlets in New York City and senate offices in Washington, D.C., in September and October 2001. These four recovered envelopes were postmarked at the USPS Trenton Processing and Distribution Center (Trenton PDC) in New Jersey.

On October 18, cutaneous anthrax was confirmed in a New Jersey postal worker. This prompted the closure of the Trenton PDC and initiation of an investigation in New Jersey. The objectives of the investigation were to determine the extent of the anthrax outbreak in New Jersey, assess potential sources of *B. anthracis* exposure, and prevent additional cases by developing and implementing control measures.

Methods

Case Definitions

In this multistate outbreak, all sites adopted the Centers for Disease Control and Prevention (CDC) case definitions for anthrax (2). A confirmed case was defined as a clinically compatible illness that was laboratory confirmed either by isolation of *B. anthracis* from an affected tissue or site, or by two supportive laboratory tests. A suspected case was defined as a clinically compatible illness with no isolation of *B. anthracis* and no alternative diagnosis, but with one positive supportive laboratory test or a clinically compatible illness epidemiologically linked to a confirmed environmental exposure to *B. anthracis*.

Supportive laboratory tests included demonstration of *B. anthracis* in a clinical specimen by immunohistochemical staining; detection of *B. anthracis* DNA by polymerase chain reaction from specimens collected from an affected tissue or site; or the presence of anti-protective antigen immunoglobulin G (anti-PA IgG) by enzyme-linked immunoadsorbent assay (3).

Case Investigations

Suspected and confirmed case-patients were interviewed about symptoms, employment, and other possible exposures, and their medical records were reviewed. Coworkers and supervisors were also interviewed. For case-patients who were USPS employees, job assignments and time sheets were reviewed, with special attention to dates when letters contain-

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ing anthrax spores were postmarked. Blood, tissue, and microbiologic samples were obtained and sent for testing. When possible, the incubation period was defined as the time between the date of likely exposure to spore-containing envelopes and the onset of symptoms.

Surveillance

Initial case finding involved investigation of potential cases reported by health-care providers, hospitals, and the public directly to the health department. Subsequently, we initiated stimulated passive hospital-based surveillance to identify additional inhalational anthrax cases (4). Infection control professionals from 61 hospitals in 15 counties in New Jersey, Pennsylvania, and Delaware, serving an area of 6.7 million residents, provided daily totals of emergency department and intensive-care unit admissions and reported all patients meeting broad clinical criteria (such as respiratory failure or febrile illness without a confirmed alternative diagnosis) for possible inhalational anthrax. Passive surveillance for both inhalational and cutaneous anthrax cases was conducted statewide in New Jersey and in parts of Pennsylvania and Delaware that are contiguous to New Jersey. Surveillance was enhanced through electronic communication with local health departments, press releases, and postings on websites of the New Jersey Department of Health and Senior Services (NJDHSS) and two New Jersey medical associations. All persons with possible anthrax identified through surveillance were followed up through telephone calls to the patients, the physicians and nurses treating them, and requests for laboratory specimens.

Exposure Assessment

To identify locations where exposures to letters containing *B. anthracis* spores might have occurred, we tracked the path of the contaminated letters through the Trenton PDC by obtaining information collected by the USPS for routine tracking and quality control. We also determined how mail flows to and from the PDC as it is brought from and delivered to other postal facilities and to the public.

Attack Rates

We reviewed the time sheets and specific work locations of the PDC employees working on the night of October 9, when the letters destined for Washington, D.C. were sorted. The number of employees working on this shift and the number of employees working on subsequent shifts were determined by review of available records and interviews with the PDC postmaster. Some records remained unavailable for review because the PDC was closed. We calculated attack rates for inhalational anthrax by dividing the number of cases by the total number of employees in the specified area.

Environmental Sampling

Initial sampling, conducted October 18–19, focused on the identified path of the letters in the Trenton PDC and public access areas of the PDC. When samples taken from areas along the path of the letters were found to be positive for *B. anthracis* on the following day, we developed a sampling strategy to evaluate the extent of contamination in the building and further characterize the risk to postal employees and visitors.

During October 21–November 9, sampling was conducted in a wider horizontal distribution around the areas of the initial positive samples and vertically upward toward the ceiling of the PDC. Sampling was performed on machinery located beyond the original path of the letters, the ventilation system, lookout galleries (enclosed elevated corridors), administrative areas on the mezzanine level, and the roof rafters. Sampling techniques included swab sampling with sterile moist swabs to collect settled dust and vacuum sock sampling with portable HEPA-filtered vacuum to collect surface dust over large areas (5).

Other mail facilities in New Jersey through which the recognized contaminated letters could have passed were identified and sampled. Most samples from these facilities were collected from areas where the initial mail-sorting activities were conducted. Additional samples were collected from customer areas, receiving bins of indoor mailboxes, cleaning equipment, loading docks, ventilation systems, computer work stations, and at least one delivery vehicle from each site. After the identification of cutaneous anthrax in an office worker who was not a PDC employee, sampling was performed at this case-patient's workplace and home; the focus was on areas where mail might have been placed or opened.

Laboratory

B. anthracis screening identification of human and environmental samples was performed at the NJDHSS Public Health and Environmental Laboratories according to Bioterrorism Laboratory Response Network Level A and B protocols, with modifications to enhance the recovery rate of spores from environmental samples (6,7). Agar slants with isolates identified as *B. anthracis* by the Public Health and Environmental laboratories were sent to CDC's Anthrax Laboratory, where identification of *B. anthracis* was confirmed by standard microbiologic procedures and the Laboratory Response Network testing algorithm (6–8). Antimicrobial susceptibility patterns were determined for *B. anthracis* isolates by using National Committee for Clinical Laboratory Standards breakpoints for staphylococci (9). Isolates of *B. anthracis* recovered from clinical specimens and environmental samples were typed to determine their genetic relatedness by using multiple-locus variable-number tandem repeat analysis (MLVA) (10).

Intervention

Postexposure prophylaxis was made available to potentially exposed persons pending results of environmental testing. We recommended continuation of postexposure prophylaxis for a total of 60 days for persons considered to be at risk for inhalational anthrax (11). A series of three postexposure prophylaxis clinic periods, each involving several sessions, were organized at two local hospitals. Seven or 10 days

of antibiotics were dispensed at the initial clinic, and 25 days of antibiotics were dispensed at each of the two follow-up clinics. Hospital staff were available for consultation with persons who could not attend the formal clinics. At the initial clinic, ciprofloxacin was provided. The recommended antibiotic for postexposure prophylaxis was changed to doxycycline for the two follow-up clinics, after testing showed the *B. anthracis* isolates were susceptible to doxycycline (12). Antibiotics were obtained from the National Pharmaceutical Stockpile.

Employees who did not attend the clinics were contacted by telephone and encouraged to come to the clinic. To promote adherence, fact sheets and a newsletter were developed and distributed, reminders for postexposure prophylaxis clinics were posted at work sites, and weekly meetings were held with USPS management and representatives from each of the four postal unions. A health education team conducted focus groups with postal employees and conducted a health education campaign.

Results

Demographic and Clinical Characteristics of Cases

From October 18 to October 24, six persons with anthrax were identified in the New Jersey area, including three with confirmed cutaneous anthrax, one with suspected cutaneous anthrax, and two with confirmed inhalational anthrax (Table 1). Their median age was 44 years (range 35–56 years); four were women. Five were USPS employees; four worked at the Trenton PDC, and one was a mail carrier at the West Trenton post office. The sixth case-patient was a bookkeeper at a Hamilton Township, New Jersey, office.

The incubation period was 5–9 days (median 8 days) for the three cutaneous cases whose exposure date could be estimated, and 5 and 6 days for the two inhalational cases. The dates of onset were clustered: two case-patients had onset of symptoms 8 and 9 days after the letters sent to New York City were processed at the Trenton PDC on September 18, and four case-patients had onset of symptoms 5–6 days after the letters sent to Washington, D.C., were processed on October 9 (Figure 1). Five of the patients were hospitalized—both persons with inhalational anthrax and three persons with cutaneous anthrax. No case-patients died. Demographic and clinical descriptions of the New Jersey case-patients are summarized in Tables 1–3 and presented in detail elsewhere (1,12–14).

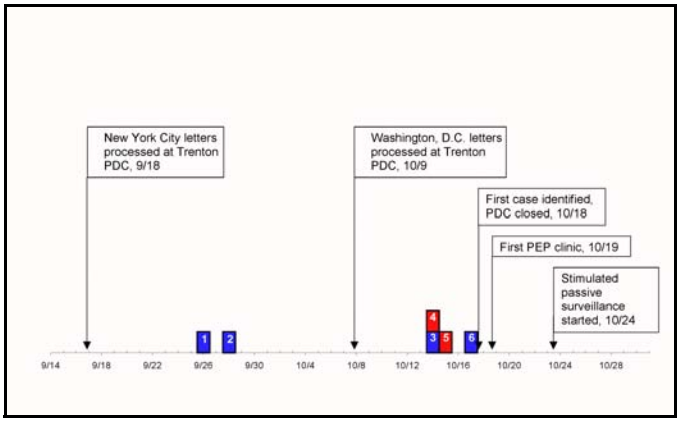

Figure 1. Timeline of events during bioterrorism-related anthrax outbreak, New Jersey, September–October, 2001. Red box = I case-patient with onset of inhalational anthrax; blue box = I case-patient with onset of cutaneous anthrax.

Surveillance

Surveillance was initiated on October 24, and from October 24 to December 17, 2001, hospital infection control practitioners reviewed 240,160 emergency department visits and 7,109 intensive-care unit admissions. Four hundred sixty-four patients who met initial criteria for possible inhalational anthrax were reported to the NJDHSS; 214 (46%) required additional follow-up to rule out inhalational anthrax. Ninety-eight patients with suspicious cutaneous lesions were reported; 26 (27%) were assessed further to rule out cutaneous anthrax. Anthrax was ruled out in all patients; no additional cases were identified (4).

Exposure Assessment and Mail Flow

The Trenton PDC occupies 281,387 square feet (approximately 7 million cubic feet) and is divided into a mail-

Table 1. Characteristics of New Jersey case-patients in the bioterrorism-related anthrax outbreak, September–October 2002

Characteristic	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Sex	Female	Male	Male	Female	Female	Female
Age (yrs)	45	39	35	56	43	51
Cutaneous/ inhalational	Cutaneous	Cutaneous	Cutaneous	Inhalational	Inhalational	Cutaneous
Postal worker	Yes	Yes	Yes	Yes	Yes	No
Employed at Trenton PDC[a]	No	Yes	Yes	Yes	Yes	No
Date of illness onset	9/28	9/26	10/14	10/14	10/15	10/17
Incubation period	9 days[b]	8 days	5 days	5 days	6 days	Unknown
Hospitalized	Yes	No	Yes	Yes	Yes	Yes
Survived	Yes	Yes	Yes	Yes	Yes	Yes

[a]PDC, postal distribution center.
[b]Assuming exposure on 9/19.

Table 2. Initial clinical findings in four patients with bioterrorism-related cutaneous anthrax, New Jersey, September–October 2001[a]

Clinical finding	No. of cases with clinical finding
Physical findings	
Edema surrounding skin lesion	4/4
Black eschar	2/4
Lesion associated with pustules or vesicles	2/4
Tender	2/4
Pruritic	1/4
Laboratory results	
Blood culture positive for *Bacillus anthracis*	1/4[b]
Blood or tissue positive for *B. anthracis* by PCR	2/4
IHC staining positive for *B. anthracis*	3/4[c]
Convalescent-phase serum[d]: anti-PA IgG antibodies present ("reactive serology")	4/4
Initial diagnosis	
Cellulitis	3/4
Insect bite	1/4

[a]IHC, immunohistochemical staining; PCR, polymerase chain reaction; anti-PA IgG, anti-protective antigen immunoglobulin G.
[b]Only 1/4 patients with cutaneous anthrax had blood cultures drawn before the initiation of antibiotic therapy. This was the one patient with a blood culture positive for *B. anthracis.*
[c]The 4th patient did not have tissue available for IHC staining.
[d]Convalescent-phase serum is serum drawn at least 14 days after symptoms begin.

processing area and administrative and public access areas. Approximately 2 million pieces of mail are processed through the facility each day. The recognized spore-containing letters destined for New York City and Washington, D.C., took similar paths as they were processed through the facility. The letters received a barcode on one of three advanced facer canceller system machines (AFCS) and were then sorted through one of two delivery barcode sorters (DBCS 70 and 71), high-speed machines that read the barcode and sort approximately 30,000 letters per hour into bins according to destination (Figure 2). The letters destined for New York City were sorted through DBCS 70 or 71 in the late afternoon of September 18. Both letters destined for Washington, D.C. were processed in the late afternoon of October 9 through AFCS 3 within approximately 15 minutes of each other, followed by sorting on DBCS 70 within 2 minutes of each other. After sorting, the letters were packed into trays in the packing area and loaded onto mail trucks (Figure 2).

In general, mail that receives the Trenton postmark at the Trenton PDC comes from one of 50 local post offices in central New Jersey, or it is dropped off in a mailbox at the Trenton PDC. We could not determine the source of the letters containing *B. anthracis*. After processing at the Trenton PDC, mail with a Trenton postmark follows one of three routes:1) it is returned to one of the 50 local post offices for local delivery; 2) it is transferred to one of 12 other PDCs in New Jersey, Philadelphia, or Delaware; or 3) it is routed through the transfer facility in Carteret, New Jersey or the airmail center in Newark en route to destinations throughout the world. We confirmed that the recognized spore-containing letters were routed through the Carteret transfer facility en route to their destinations in New York City and Washington, D.C. At the Carteret transfer facility, mail is not unwrapped or handled; it remains in tubs that are transferred from one truck to another.

Potential Case Exposures

Case-patient 1 (Table 1) was a mail carrier at the West Trenton post office, which sends and receives its mail through the Trenton PDC. She never worked at the Trenton PDC and did not visit that facility. The mail that this carrier delivered on

Table 3. Clinical findings in two patients with bioterrorism-related inhalational anthrax, New Jersey, September–October 2001[a]

Clinical finding	Case 1	Case 2
Past medical history	Transient ischemic attack	None
Smoking status	Nonsmoker	Nonsmoker
Initial symptoms	Fever, chills, vomiting, diarrhea	Fever, chills, vomiting, dry cough, headache
Signs at ER visit	Fever: temp=38.4°C; Tachycardia: HR=120/min; Hypoxia: arterial paO2=58 (RA)	Fever: temp=38.4°C; Tachycardia: HR=120/min; Hypoxia: SaO2=92% (RA)
Chest x-ray	Infiltrate, pleural effusion	Infiltrate, pleural effusion
Hospital course	Re-accumulating hemorrhagic pleural effusions	Re-accumulating hemorrhagic pleural effusions
Laboratory results		
Blood culture	Negative (before start of antibiotics)	Negative (after 2 days of antibiotics)
Blood positive for *Bacillus anthracis* by PCR	Yes (before start of antibiotics)	No (after 2 days of antibiotics)
IHC staining of pleural fluid Cytology	Positive for *B. anthracis* cell wall Positive for *B. anthracis* capsule	Positive for *B. anthracis* cell wall Positive for *B. anthracis* capsule
Convalescent-phase serum[b]	Anti-PA IgG antibodies present	Anti-PA IgG antibodies present

[a]ER, emergency room; HIC, immunohistochemical; PCR, polymerase chain reaction; Anti-PA IgG, anti-protective antigen immunoglobulin G.
[b]Convalescent-phase serum is serum drawn at least 14 days after symptoms begin.

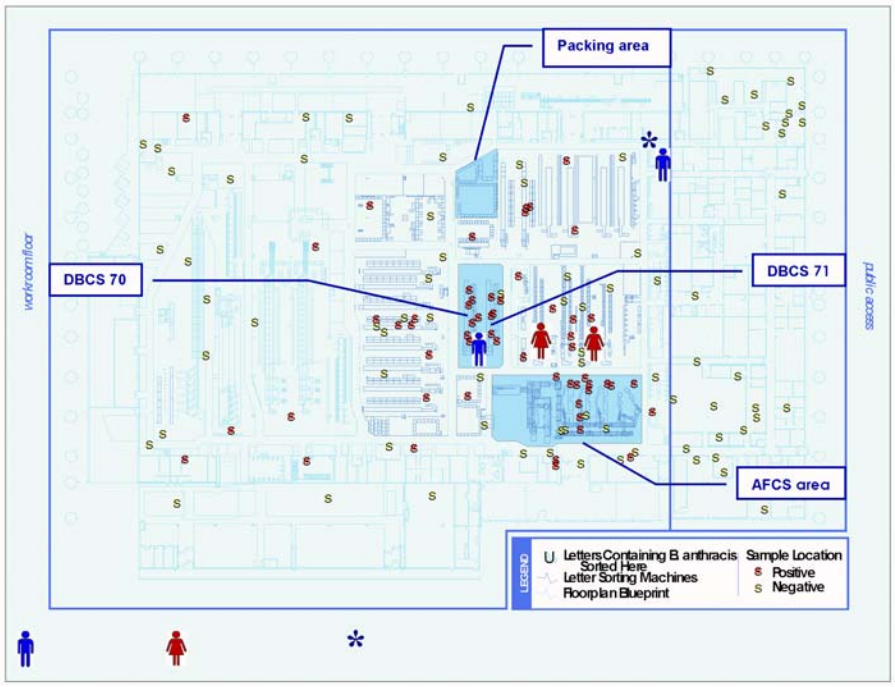

Figure 2. Floor map of the Trenton Postal Distribution Center in Hamilton Township with locations of environmental samples taken October–November, 2001, and work stations of New Jersey case-patients on dates when letters containing *Bacillus anthracis* were sorted. Blue man = male, cutaneous anthrax; red woman = female, inhalational anthrax.
*Machine mechanic worked throughout the mail-sorting area the night the letters containing *B. anthracis* destined for New York were sorted.

September 19 had been sorted at the PDC on September 18 on the same machines that had sorted the New York City letters earlier that day. On September 28, 10 days later, cutaneous anthrax developed in this mail carrier.

Case-patient 2 (Table 1) was a machine technician. He worked on September 18, 2001, when the letters to the New York City media outlets were sorted. This technician circulated throughout the letter-sorting area, was responsible for maintenance and repair of the high-speed sorters, and used compressed air to blow out dust and debris from the machines. Cutaneous anthrax developed in this man 8 days later.

Case-patient 3 (Table 1) was working on October 9, when the letters containing *B. anthracis* bound for Washington, D.C. were processed. Although he began his shift working in a different area of the facility, he later moved to operate the DBCS 70 that had sorted the letters containing *B. anthracis* earlier that evening (Figure 2). Cutaneous anthrax developed 5 days later.

The two New Jersey case-patients with inhalational anthrax (Case-patients 4 and 5, Table 1) were also working on the night of October 9. They stood side by side at the input subsystem sorters, machines located next to the AFCS and the DBCS that sorted the *B. anthracis*–containing letters (Figure 2). Inhalational anthrax developed in these case-patients 5 and 6 days later, respectively.

Case-patient 6 was a bookkeeper at a Hamilton Township accounting firm; she did not visit the Trenton PDC. However, mail delivered to both her home and workplace came directly from the Trenton PDC without passing through an intermediate local post office. The bookkeeper's onset of cutaneous anthrax was October 17, eight days after the Washington, D.C. destined letters were processed at the Trenton PDC.

Attack Rates for Inhalational Anthrax after Exposure to Washington, D.C.–Destined Letters

The two case-patients with inhalational anthrax (Case-patients 3, 4, Table 1) were identified among 750 Trenton PDC employees who worked in the processing area of the facility during or after the letters addressed to Washington, D.C. were processed on October 9 (overall attack rate 0.25%). The two persons with inhalation anthrax were among 170 who worked in the sorting area on the October 9 shift when the letters transited, yielding an attack rate of 1.2% among sorting area workers.

Environmental Sampling

Of the 137 samples obtained at the Trenton PDC, 57 (42%) were positive for *B. anthracis* (Figure 2, Table 4). Positive samples were located throughout the facility, including samples taken from rafters as high as 25 feet above the plant floor and samples from the ventilation system (Figure 2). Twenty-five (83%) of 30 samples were positive in the area where the letters containing *B. anthracis* were sorted. Positive samples were identified from the machines at which Case-patients 4 and 5 worked and from the sorting machine that processed mail destined for the workplace and home of Case-patient 6.

In addition to the samples collected at West Trenton post office, we obtained a mean of 18 samples (range 4–27 samples) from each of the other 49 local post offices. Five of the local post offices had one positive sample each. The positive sample in each facility came from an area where mail from the Trenton PDC is deposited. One of the samples was obtained underneath a sorting machine, three were obtained from mail containers or the place where mail containers are stored, and one was from a bin inside a mailbox outside the post office. All 57 samples collected from the West Trenton post office

Table 4. Environmental sampling results of bioterrorism-related anthrax outbreak, New Jersey, October–November 2001

Site	No. of samples	Results
Trenton Postal Distribution Center		
Entire facility	137	57 (42%) positive
Letter-sorting area	30	25 (83%) positive
Customer service area (public area)	20	0 positive
Carteret Transfer Facility	14	0 positive
West Trenton Post Office	57	0 positive
Other 49 local post offices	983	5 (0.5%) positive[a]
		1/72 positive PO[b] #1 (1.4%)
		1/19 positive PO #2 (5.3%)
		1/15 positive PO #3 (6.7%)
		1/18 positive PO #4 (5.6%)
		1/24 positive PO #5 (4.2%)
Bookkeeper's home	5	0 positive
Bookkeeper's workplace	21	1 (4.7%) positive

[a]One each at five distinct facilities.
[b]PO, post office

(where Case-patient 1 worked) were negative. All 14 samples from the Carteret facility were negative.

Of 21 samples collected from the workplace of Case-patient 6, one grew *B. anthracis*. This sample was obtained from a tray near the receptionist's desk that held delivered and outgoing mail. None of the samples collected from the home of Case-patient 6 were positive, including samples collected from her mailbox and areas where she stored and opened her mail.

Of the 10 environmental isolates typed by MLVA (4 from locations throughout the Trenton PDC, 5 from the local post offices, and 1 from the workplace of Case-patient 6), all were indistinguishable from clinical isolates.

Interventions

We recommended 60 days of postexposure prophylaxis for 1,069 employees of the Trenton PDC, as well as for persons who visited the facility and spent >1 hour on the plant floor from September 18 (the date the first letter containing *B. anthracis* was processed in the Trenton PDC) to October 18, 2001 (the date the facility was closed). Beginning October 20, a total of 885 (83%) Trenton PDC postal workers were provided with the full 60-day course of postexposure prophylaxis. Of the 184 (17%) postal workers who did not receive 60 days of antibiotics, 29 (3%) did not receive any antibiotics, 40 (4%) came to the initial clinic only; and 115 (11%) came to the initial and first follow-up clinics. Most postal workers (1,032 [97%]) obtained their antibiotics from Hospital A; 37 (3%) obtained antibiotics from their private physicians.

Three hundred twenty-four visitors to Trenton PDC went to Hospital A (n=175), Hospital B (n=129), or their private physicians (n=20) for prophylaxis. Of these, 206 (64%) received 60 days of antibiotics, 85 (26%) received <60 days, and 33 (10%) did not receive any antibiotics.

Discussion

In New Jersey, *B. anthracis* spores contained in envelopes processed on high-speed mail sorting machines were the source of two cases of inhalational anthrax, four cases of cutaneous anthrax, widespread contamination of the Trenton PDC, and cross-contamination of other letters, equipment, and facilities. Several aspects of the New Jersey outbreak provide insights into how these *B. anthracis* spores were distributed in the environment, the clinical signs and symptoms they caused, and the challenges to public health that arose in the setting of intentional *B. anthracis* contamination.

Envelopes containing *B. anthracis* were handled at the Trenton PDC in a limited area of the facility: they passed through a small number of the many machines used to handle letters. Yet environmental sampling found evidence of spores throughout the facility, including on nearly all of the sorting machines, in the ventilation system, and in the rafters high above the plant floor. These findings are consistent with recent experiments indicating that spores deposited on high-speed sorting machines from the passage of *B. anthracis*–containing envelopes can be readily aerosolized or dispersed through the air and are capable of being carried for considerable distances (15).

Despite evidence of distribution of spores throughout the facility, the epidemiologic investigation demonstrated limited disease. The attack rate among Trenton PDC workers for inhalational anthrax was low, despite the potential for ongoing exposure during the 9 days between the afternoon the letters bound for Washington, D.C. were processed and the day the facility was closed. The two workers in whom inhalational anthrax developed stood next to one another when the letters containing *B. anthracis* were sorted: they worked on machines next to the sorters that processed these letters. Symptoms developed in these workers within 1 day of each other. These findings are consistent with an exposure to a local plume of aerosolized spores during or soon after the passage of the letters. Such a plume could have been produced by air circulation patterns in the vicinity or when compressed air was used to blow out or clean a nearby machine that had processed the letters. We had no means of assessing individual exposure to explore this hypothesis further. For example, all the >900 nasal swabs collected from Trenton PDC workers were negative for *B. anthracis* but were collected at least 10 days after the last known letters were sorted in the facility, perhaps too long after potential exposure to be useful indicators.

The Trenton PDC is the only facility identified in which exposure to letters bound both for New York City and Washington, D.C. occurred, allowing for comparison between the outcomes of these exposures. In New Jersey, only cutaneous anthrax occurred after the letters to New York City were sorted. Although we cannot exclude the possibility that the

cases that occurred in temporal association with processing of the Washington, D.C.–destined letters might have been acquired from exposure to the New York City–destined letters, both inhalational and cutaneous anthrax most likely occurred in New Jersey after exposure to the letters to Washington, D.C. Although only inhalational cases were reported in Washington, D.C., these findings are consistent with the predominant forms of anthrax that occurred following exposures to these letters in New York City and Washington, D.C. (12,14). Many factors, including differences in powder or other characteristics in the contaminated letters, as well as differences in environmental or other conditions at the various sites, might account for differences in disease associated with the exposures to the New York City– and Washington, D.C.–destined letters. Ongoing studies of spore and envelope characteristics and aerosol formation during routine mail processing activities might provide further insight.

Two of the six New Jersey cases occurred in persons who did not work at the Trenton PDC and would not have had a direct exposure to a recognized spore-containing letter at any point in the known letter path. In both circumstances, we demonstrated the opportunity for exposure to mail that could have been cross-contaminated when spores deposited in sorting machines or on other equipment were transferred to envelopes subsequently processed in the facility. Although these cases could possibly have resulted from unrecognized direct exposure to envelopes containing *B. anthracis*, we consider exposure to cross-contaminated envelopes to be the probable source of these two cases. Consideration of the potential number of envelopes that might have been cross-contaminated in this fashion gives an appreciation of the rarity of disease from exposure to cross-contaminated envelopes. During the 9-day period after processing of the letters bound for Washington, D.C., before the facility was closed, at least 2 million letters could have been sorted through the same machine that sorted the spore-containing envelopes, and an estimated 18 million pieces of mail would have been processed through the facility. Yet only one case of cutaneous anthrax occurred among the many thousands of USPS employees who handled mail that had passed through the Trenton PDC, and only one case was identified among the many millions of recipients of such envelopes living in our surveillance area. Thus, the risk of anthrax from cross-contamination, while not absent, appears to be quite low.

Given the urgent public health actions that followed the identification of each new case—from facility closures to recommendations for postexposure prophylaxis for hundreds—surveillance played a crucial role in this investigation. We continued surveillance for 8 weeks after the last case had been identified because the outer limit of the incubation period was poorly defined, the extent that mail and other postal facilities had been cross-contaminated was unknown, and there was a possibility that additional *B. anthracis*–containing letters would be posted or other terrorist events would occur. Thus, surveillance was pivotal in demonstrating that the scope of the outbreak was limited to the original cases identified and that the risk to the general population was low. Surveillance also provided a level of assurance that other attacks were not occurring in the area and confirmed that additional public health control measures were not needed. Surveillance also enabled NJDHSS and CDC officials to maintain timely and frequent communication with the health-care community, defined a clear role for health-care providers and hospitals in the response efforts, and provided assurance and consultation to the health-care community and the public.

Effective and frequent communication among postal workers, hospital health-care workers, and NJDHSS and CDC staff members also contributed to the high rate of initiation and completion of postexposure prophylaxis in New Jersey. Some studies have indicated that creating realistic patient expectations about side effects and enhancing patient understanding of illness and treatment promote adherence (16,17). The three postexposure prophylaxis clinics held in New Jersey enabled postal workers to ask questions about anthrax, antibiotic regimens, adverse effects associated with taking the antibiotics, and ways to make taking prophylaxis more tolerable. Close patient follow-up also promotes adherence (16,17), especially when the course of treatment is long. In New Jersey, we made telephone calls to postal workers who did not attend a clinic, and hospital staff were available to see these workers for medication refills outside the formal clinics.

The New Jersey investigation highlighted unprecedented and unanticipated challenges to public health posed by the intentional release of a pathogenic biologic agent. An urgent public health response led to the rapid development of diagnostic and environmental sampling methods that were refined as the investigation progressed. The implementation of postexposure prophylaxis measures required the development of a large-scale medication delivery infrastructure. Health communication messages were revised daily and often required communicating the uncertainty of risk through the lay media. The possibility of further attacks with anthrax spores or other agents of terrorism remains. Continued vigilance and close cooperation among the various health, law enforcement, and other groups and agencies, as well as continued support of efforts to rebuild and update the public health infrastructure, are needed to protect the public's health. This relatively limited bioterrorism attack required considerable resources and time from public health, health-care providers and hospitals, and law enforcement. Further evaluation of the New Jersey and other anthrax bioterrorism investigations may prove helpful in developing responses to future attacks.

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References

1. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–93.
2. Centers for Disease Control and Prevention. CDC case definition of anthrax and summary of confirmed cases. Available from: URL: http://www.bt.cdc.gov/DocumentsApp/Anthrax/10212001PM.asp
3. Greene CM, Pinner RW, Sejvar J**,** Dull P, Jernigan J, Stephens D, et al. Rapid adaptation of a serologic assay for the bioterrorism-related anthrax outbreak—October-December 2001. In: Abstracts of the International Conference on Emerging Infectious Diseases 2002; Atlanta, Georgia; 2002 Mar 24–27. Atlanta, Georgia: Centers for Disease Control and Prevention; 2002.
4. Tan CG, Sandhu HS, Crawford DC, Redd SC, Beach MJ, Buehler J, et al. Surveillance for anthrax cases associated with contaminated letters, New Jersey, Delaware, and Pennsylvania, 2001. Emerg Infect Dis 2002;8;1073-7.
5. Centers for Disease Control and Prevention. Procedures for collecting surface environmental samples for culturing *Bacillus anthracis*. Available from: URL: http://www.bt.cdc.gov/DocumentsApp/Anthrax/11132001/Final42.asp
6. Turnbull PC. Definitive identification of *Bacillus anthracis*— a review. J Appl Microbiol 1999;87:237–40.
7. Khan A, Morse S, Lillibridge S. Public health preparedness for biological terrorism in the USA. Lancet 2000;356:1179–82.
8. Logan NA, Turnbull PC. *Bacillus* and recently derived genera. In: Murray PR, Baron EJ, Pfaller M, Tenover FC, Yolken RH, editors. Manual of clinical microbiology. 7th edition. Washington: American Society for Microbiology; 1999. p. 357–69.
9. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Wayne (PA):The Commettee; 2001. 11th informational supplement M100-S11.
10. Keim P, Price LB, Klevytska AM**,** Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships with *Bacillus anthracis*. J Bacteriol 2000;182:2928–36.
11. Centers for Disease Control and Prevention. Notice to readers: ongoing investigation of anthrax—Florida, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:877.
12. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
13. Jernigan JA, Stephens DS, Ashford DA**,** Omenaca C, Topiel MS, Galbraith M, et. al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
14. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
15. Dull PM, Wilson KE, Kournikakis B, Whitney EAS, Boulet CA, Ho JYW, et al. Bacillus anthracis aerosolization associated with a contaminated mail sorting machine. Emerg Infect Dis 2002;8;1044-7.
16. Erwin J, Peters BS. The critical relationship between compliance and the management of infectious diseases. Clin Microbiol Infect 1998;4:275–8.
17. Sanson-Fisher R, Bowman J, Armstrong S. Factors affecting nonadherence with antibiotics. Diagn Microbiol Infect Dis 1992;15:103S–9S.

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"I have not failed. I've just found 10,000 ways that won't work."
–Thomas Alva Edison

Bioterrorism-Related Anthrax: International Response by the Centers for Disease Control and Prevention

Christina S. Polyak,* Jonathan T. Macy,* Margarita Irizarry-De La Cruz,* James E. Lai,* Jay F. McAuliffe,* Tanja Popovic,* Segaran P. Pillai,† Eric D. Mintz,* and the Emergency Operations Center International Team[1]

After reports of the intentional release of *Bacillus anthracis* in the United States, epidemiologists, laboratorians, and clinicians around the world were called upon to respond to widespread political and public concerns. To respond to inquiries from other countries regarding anthrax and bioterrorism, the Centers for Disease Control and Prevention established an international team in its Emergency Operations Center. From October 12, 2001, to January 2, 2002, this team received 130 requests from 70 countries and 2 territories. Requests originated from ministries of health, international organizations, and physicians and included subjects ranging from laboratory procedures and clinical evaluations to assessments of environmental and occupational health risks. The information and technical support provided by the international team helped allay fears, prevent unnecessary antibiotic treatment, and enhance laboratory-based surveillance for bioterrorism events worldwide.

Immediately following reports of the intentional release of *Bacillus anthracis* in the United States in October 2001, public health professionals around the world were called upon to respond to widespread political and public concerns. Specific threats, hoaxes, and incidents in other countries directly affected U.S. institutions and citizens, as well as expatriate U.S. government employees, businessmen, journalists, and travelers. The Centers for Disease Control and Prevention (CDC) established an international team in its Emergency Operations Center to respond to inquiries from other countries regarding anthrax and bioterrorism.

Methods

The international team included physicians, microbiologists, epidemiologists, and other public health officials with expertise in international affairs and infectious diseases. Team members were fluent in several languages. From October 12, 2001, to January 2, 2002, the team provided rapid feedback and support in response to requests for assistance on bioterrorism-related topics. The team was available for consultation by telephone and e-mail, 24 hours a day, 7 days a week. The team electronically disseminated documents on anthrax and bioterrorism preparedness and collaborated with the World Health Organization (WHO) and its regional offices to facilitate exchanging relevant information.

Requests for assistance were classified into four general categories: laboratory-related issues, general bioterrorism information, environmental and occupational concerns, and bioterrorism preparedness. Depending on the nature of the request, the team sought assistance from other CDC experts.

The level of support provided to various countries for specific requests was divided into two categories: high or medium. High, or technical, support included one or more of the following: testing clinical and environmental or nonclinical specimens and isolates, arranging for specimens and isolates to be tested at a reference laboratory, coordinating with CDC staff in-country to provide on-site consultation and assistance, and providing reagents for performing microbiological tests. Medium, or informational support, included telephone or e-mail consultation regarding bioterrorism, laboratory methods, and preparedness.

Results

The international team received 130 requests for assistance from 70 countries and 2 territories during the period October 12, 2001, to January 2, 2002. An average of 3.2 requests per day (with a peak of 9 requests on October 19) were received by e-mail (55.4%) and telephone (44.6%) (Figure 1). Forms of support provided to other countries included consultation regarding laboratory methods for isolation and identification of *B. anthracis*, clinical and epidemiologic support, and policy and preparedness. Of the 130 requests, 54 (41.5%) were laboratory related; 51 (39.2%) were general requests for bioterrorism information; 14 (10.8%) were for environmental or

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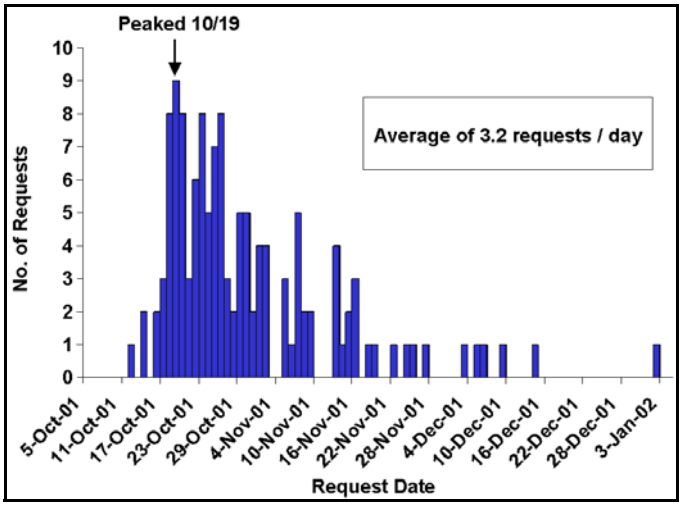

Figure 1. Requests for assistance to international team of the Emergency Operations Center, October 12, 2001–January 2, 2002 (n=130).

occupational health guidelines; and 11 (8.5%) concerned developing bioterrorism-preparedness plans. Ninety-three (71.5%) of the requests were from persons or agencies affiliated with Ministries of Health; 15 (11.5%) were from other public health or medical professionals; 13 (10.0%) were from private citizens; and 9 (6.9%) were from international organizations such as WHO and the Pan American Health Organization. Requests were not evenly distributed by region. Europe and Latin America/the Caribbean each accounted for 25.4% of the total requests, followed by Asia (22%) and Africa (15%).

Of the 70 countries and 2 territories, 55 (76.4%) received informational support or telephone or e-mail consultation regarding bioterrorism events or preparedness (Figure 2). The remaining 17 (23.6%) received a high or technical level of support, including testing specimens at a Laboratory Response Network member reference laboratory (n=12). Digital images of suspected *B. anthracis* isolates and cases were submitted by e-mail, which enabled laboratory and clinical experts to review images of suspected cases worldwide and provide rapid guidance (Figure 3). All isolates and cases from images sent were subsequently found to be negative.

Four isolates from outside the United States were confirmed as *B. anthracis*. Three of these isolates were cultured from mail sent by the U.S. Department of State to U.S. embassies in Lima, Peru (two), and Vienna, Austria (one). These three isolates were indistinguishable from all other U.S. outbreak isolates by molecular subtyping by multilocus variable number of tandem repeats typing. An additional isolate, recovered by the Chilean National Institute of Public Health from a letter to a private physician in Chile, was a different subtype from those in the U.S. outbreak. The source of this isolate is being investigated.

The team's active role concentrated on information dissemination and collaboration with WHO. Documents on anthrax and bioterrorism were prepared and disseminated electronically to all CDC international assignees (41 countries), to epidemiologists and laboratorians affiliated with the Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET, 33 countries), and to the WHO Global Salmonella Surveillance List Serve (Global Salm-Surv, 106 countries). Electronic dissemination allowed rapid distribution and availability of contact information for the team. In addition, the Morbidity and Mortality Weekly Report (MMWR) published a short statement about the team's activities that described assistance to other countries and directed questions regarding bioterrorism-related issues outside the United States to the team (1). Collaboration with WHO regional offices included the development and support of a training course on the management of suspected exposures to anthrax spores (2). Representatives from 14 countries attended the course, which was conducted in Bangkok in December 2001 and was sponsored by the CDC International Emerging Infections Program

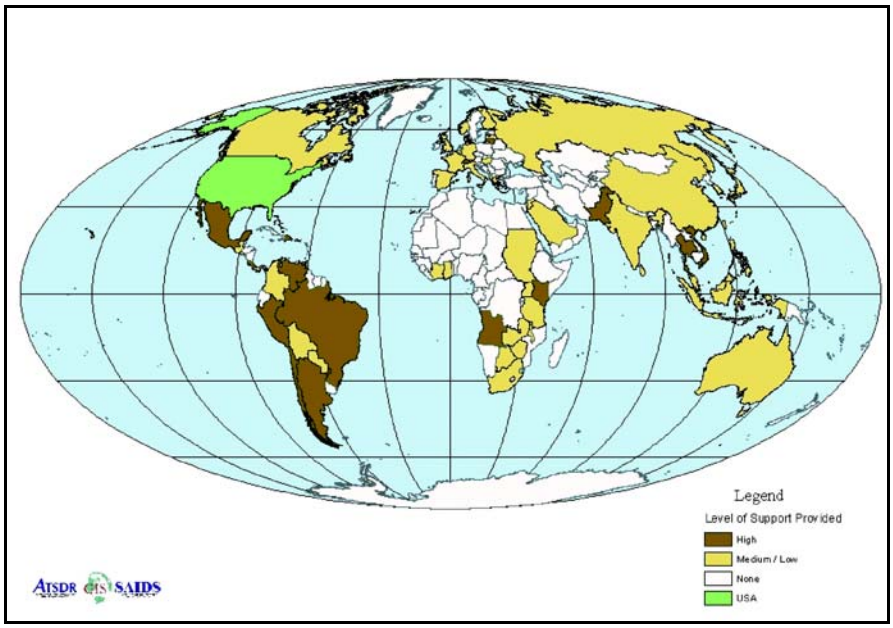

Figure 2. International response: level of support provided.

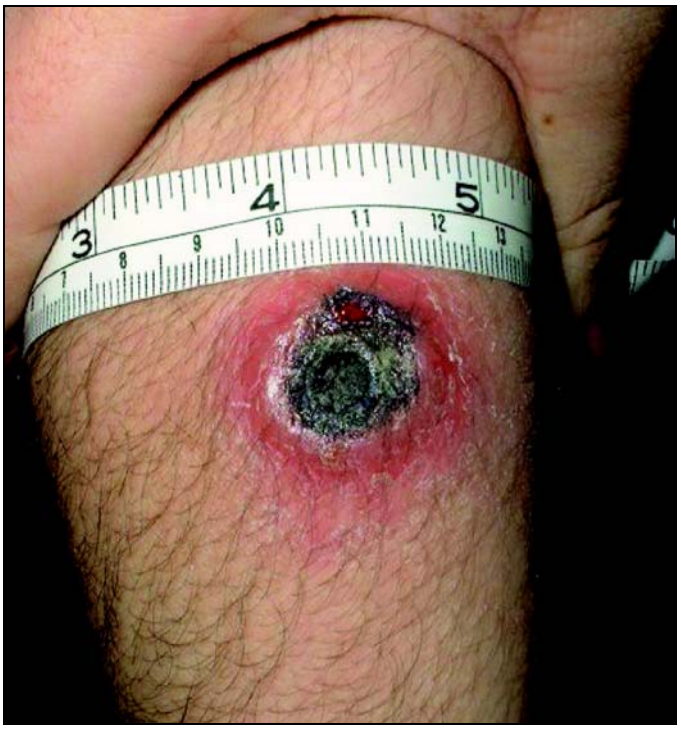

Figure 3. Suspected cutaneous anthrax lesion from a patient in the United Kingdom. Photos like this, transmitted by e-mail, enabled clinical experts to review images of suspected cases worldwide and provide rapid assistance.

in Thailand and the WHO headquarters Southeast Asia Regional Office. In addition, the international team worked with WHO to develop a database of laboratories capable of serving as anthrax reference laboratories in various countries throughout the world.

Discussion

Any suspected bioterrorism event has immediate global implications, no matter who the intended target or where the event occurs. This global impact is particularly true for communicable diseases such as smallpox. However, because of international trade, travel, and social connectedness, the same principle applies to less easily transmitted communicable diseases such as anthrax. More than 4,000 threat letters were tested in public health laboratories in Europe in the month after the first report of intentionally contaminated mail in the United States, and all surveyed national public health institutions took extraordinary measures to improve bioterrorism preparedness (3).

In countries throughout the world, threat letters caused a shift in resources from traditional public health concerns to national security concerns. This shift represents a particular challenge for developing countries with chronically scarce resources for public health. Therefore, additional resources, particularly for the health sector of developing countries, are needed to address future threats. In many countries, strengthening the public health surveillance and response capacity for naturally occurring emerging infectious diseases is the most efficient means to provide a critical early warning system for intentionally released biologic agents and a defense against their further spread.

Public health agencies need to be able to exchange information rapidly across international borders to keep pace with events and make critical medical and public health decisions. Public health agencies must also keep pace with worldwide media coverage to minimize the potential for misguided public reaction. In the United States and other countries, many persons who were exposed to suspected anthrax-containing materials were told to not start or to discontinue antibiotic chemoprophylaxis after anthrax exposure was ruled out by testing at public health reference laboratories and by further epidemiologic investigations (4). Information and technical support provided by the international team helped allay fears, prevent unnecessary antibiotic treatment, and enhance laboratory-based surveillance for bioterrorism events worldwide.

The operations of the international team were not without difficulty. Responding rapidly in different languages to countries in different time zones proved to be a challenge. In addition, the team was not always able to provide rapid technical assistance because of the need for review and clearance of documents containing new scientific information. Despite strict adherence to regulations governing the transport of infectious agents, shipment of suspected isolates of *B. anthracis* from laboratories in one country to reference laboratories in the United States was complicated by hesitance from shipping companies, air carriers, and national authorities. In some cases, the laboratory investigation of suspected exposures was delayed for several days while consent was sought from higher authorities and willing shipping companies and air carriers were identified.

The largest percentage of requests received by the international team were from persons or agencies affiliated with ministries of health, reflecting concern about bioterrorism issues at the national government level. On request, the team also provided specific information about the events occurring in the United States, often through referrals to publications and other materials regularly posted on the CDC website. Information provided to field epidemiology training programs through TEPHINET addressed some of these issues proactively and reduced the overall number of requests (5). Given the essential role of the public health laboratory in bioterrorism preparedness and response (6), information provided proactively to laboratories through the WHO Global Salm-Surv listserv may also have reduced the number of requests.

Rapid, reliable access to the Internet is an extremely useful tool for connecting public health agencies and laboratories and should be universally promoted. Digital cameras are an economical means of capturing clinical and laboratory images for Internet transmission and can greatly enhance communication about suspected cases or specific etiologic agents of infectious diseases. Nonclassified commercial laboratory reagents and protocols for isolating and identifying *B. anthracis* and other bioterrorism agents should be widely available to national

public health reference laboratories. Through its collaborating centers, WHO has already begun to establish a worldwide network of reference laboratories capable of isolating, identifying, and confirming bioterrorism agents; WHO will continue to play a critical role in global coordination of outbreak surveillance and response. In addition, during the World Health Assembly of May 2002, the 191 member states agreed to a resolution recognizing that a deliberate release of biological agents could have serious public health implications and jeopardize public health achievements of the past decades (7).

In the long term, strengthening the capabilities of national public health agencies and laboratories to recognize and respond to potential bioterrorist events and agents will also build capacity for recognition and response to naturally occurring outbreaks. Ensuring connectivity between these national public health agencies and reference laboratories worldwide is critical to improving global preparedness for emerging infectious diseases, whether or not they result from the deliberate release of a bioterrorism agent.

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References

1. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1008–10.
2. Simmerman JM, Kumari S, Bhatia R, Dejsirilert S, Sangsuk L, Sawanpanyalert P, et al. Strengthening public health response to anthrax in Southeast Asia. Proceedings of the 10th International Conference on Infectious Diseases 2002, March 11–14, 2002. Singapore.
3. Coignard B. Bioterrorism preparedness and response in European public health institutes. Eurosurveillance 2001;6:159–66.
4. Centers for Disease Control and Prevention. Notice to readers: interim guidelines for investigation of and response to *Bacillus anthracis* exposures. MMWR Morb Mortal Wkly Rep 2001;50:987–90.
5. Sandhu HS, Thomas C, Nsubuga P, White M, Mendlein J, Maes E, et al. Assessment of bioterrorism response capacity of applied epidemiology and training programs—2001. Proceedings of International Conference on Emerging Infectious Diseases 2002, March 24, 2002. Atlanta, Georgia.
6. Khan A, Morse S, Lillibridge S. Public health preparedness for biological terrorism in the USA. Lancet 2000:356:1179–82.
7. World Health Organization. Global public health response to natural occurrence, accidental release or deliberate use of biological and chemical agents or radionuclear material that affect health. Geneva: 55th World Health Assembly; May 18, 2002.

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Two-Component Direct Fluorescent-Antibody Assay for Rapid Identification of *Bacillus anthracis*

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A two-component direct fluorescent-antibody (DFA) assay, using fluorescein-labeled monoclonal antibodies specific to the *Bacillus anthracis* cell wall (CW-DFA) and capsule (CAP-DFA) antigens, was evaluated and validated for rapid identification of *B. anthracis*. We analyzed 230 *B. anthracis* isolates; 228 and 229 were positive by CW-DFA and CAP-DFA assays, respectively. We also tested 56 non–*B. anthracis* strains; 10 *B. cereus* and 2 *B. thuringiensis* were positive by the CW-DFA assay, and 1 *B. megaterium* strain was positive by CAP-DFA. Analysis of the combined DFA results identified 227 of 230 *B. anthracis* isolates; all 56 strains of the other *Bacillus* spp. were negative. Both DFA assays tested positive on 14 of 26 aging clinical specimens from the 2001 anthrax outbreak investigation. The two-component DFA assay is a sensitive, specific, and rapid confirmatory test for *B. anthracis* in cultures and may be useful directly on clinical specimens.

The potential use of *Bacillus anthracis* as a biological weapon has long been recognized (1–5). Recently, the profound impact of *B. anthracis* on public health was demonstrated during the bioterrorism-related anthrax outbreak in the United States (6). Rapid diagnosis played an important role during the outbreak and aided in implementing appropriate public health measures in a timely manner. Although several standard microbiologic assays are available to identify *B. anthracis* (7), they primarily lack timeliness in producing results.

Earlier studies demonstrated the advantages of immunofluorescence assays, based on polyclonal antibodies to *B. anthracis* cell-surface antigens, for identifying *B. anthracis* isolates (8) and directly evaluating clinical specimens from infected guinea pigs (9). However, the limitations of polyclonal antibodies, such as the problem of cross-reactivity with closely related *Bacillus* species known as the *B. cereus* complex (10), were also apparent. Over the past decade, monoclonal antibodies specific to the *B. anthracis* cell wall polysaccharide antigen were shown to be useful in identification of *B. anthracis* infection (11,12). Vegetative *B. anthracis* cells constitutively express the galactose/N-acetylglucosamine polysaccharide cell wall antigen (13,14). In addition, during infection or growth in nutrient-rich media in an elevated CO_2 environment, *B. anthracis* cells produce a poly-γ-D-glutamic acid capsule, which is synthesized by the products of genes located on the pXO2 plasmid (15). The capsule masks the cell wall polysaccharide (11); however, the polysaccharide becomes detectable in aging clinical samples (N. Woollen, pers. comm.).In this study, we have evaluated and validated a two-component direct fluorescent-antibody (DFA) assay, using the monoclonal immunoglobulin (Ig) M antibody EAII-6G6-2-3 against the cell wall polysaccharide antigen (CW) (12) and the monoclonal IgG antibody FDF-1B9 against the capsule antigen (CAP) (16) for rapid identification of *B. anthracis*. In addition to use on isolates, this rapid DFA assay was applied successfully to detect *B. anthracis* directly in clinical specimens from several patients with laboratory-confirmed inhalational anthrax during the 2001 bioterrorism-associated anthrax outbreak in the United States (6,17).

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Materials and Methods

Bacterial Isolates

B. anthracis Isolates (n=230)

Eighty-one *B. anthracis* isolates from different sources (human, animal, and environmental) representing broad geographic and temporal (1939–1997) diversity were selected from culture collections at the Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia. Six of these isolates were free of pXO1 or pXO2 plasmids. An additional 149 *B. anthracis* isolates, obtained from powders (n=4), 10 patients (n=26), and environmental sources (n=125) during the investigation of the U.S. bioterrorism-associated anthrax outbreak from October 5 to December 21, 2001, were included.

Other *Bacillus* spp. (n=56)

Five closely related *Bacillus* species—*B. cereus* (n=23), *B. megaterium* (n=11), *B. subtilis* (n=9), *B. thuringiensis* (n=12), and *B. mycoides* (n=1)—were selected to test the specificity of the DFA assays. Most *B. cereus* isolates (n=20) were from different sources (environmental, food, human, and animal) representing broad geographic and temporal (1957–2000) diversity.

Control Strains (n=2)

B. anthracis Pasteur (ATCC 4229) and *B. cereus* (ATCC 14579) were used as positive and negative controls, respectively, for both CW- and CAP-DFA assays. The control strains were stored at 4°C as spore suspensions in water. All other strains were kept at –70°C as spore suspensions in water or in 2.5% heart infusion broth (HIB) containing 20% glycerol. All strains were identified by standard microbiologic procedures (7), and confirmatory identification of *B. anthracis* strains was performed according to the Laboratory Response Network testing algorithm (5) using a battery of tests including the DFA assay described in this study.

Clinical Specimens

Twenty-six clinical specimens (stored at 4° for >1 month), including aerobic and anaerobic blood cultures (n=11), various body fluids (n=6), pleural fluids (n=4), lung tissues (n=3), and lymph nodes (n=2), were collected from seven patients with laboratory-confirmed inhalational anthrax from October through December 2001 (6,17,18).

Preparation of Fluorescein-Antibody Conjugates

Two monoclonal antibodies, EAII-6G6-2-3 (12) and FDF-1B9 (16), were purified by HiTrap SP Gradifrac cation exchange chromatography (Pharmacia, Peapack, NJ) to homogeneity and conjugated to fluorescein isothiocyanate (FITC), according to a standard protocol (Molecular Probes, Eugene, OR). The anti-cell wall (anti-CW FITC) and anti-capsule (anti-CAP FITC) conjugates were lyophilized in HEPES buffer (0.05 M HEPES, pH 7.0, 0.10% glycine, 0.01 M d-sorbitol, 0.15 M KCl, and 5% d-trehalose) containing 1% bovine serum albumin (Cohn Fraction V) (Sigma Chemical Co., St. Louis, MO). The working antibody solutions (50 µg/mL) were prepared in 50% glycerol in water and stored at –20°C or 4°C.

Preparation of Cell Suspensions for DFA Assays

Vegetative Cells for the CW-DFA Assay

For each control and test strain, fresh vegetative cells were grown by plating stock spore suspension (1 µL) on trypticase soy agar with 5% sheep blood (SBA) (BBL Microbiology Systems, Cockeysville, MD) and incubating aerobically overnight at 37°C. The cell suspensions were prepared by suspending one loop (1 µL) of the SBA culture in 100 µL of 10 mM phosphate-buffered saline/0.3% Tween 20, pH 7.2 (PBST) and adjusting the concentration to ~10^7 cells/mL (equivalent to a 0.5 McFarland standard).

Encapsulated Cells for the CAP-DFA Assay

For each control and test strain, encapsulated cells were grown by transferring an overnight growth of fresh vegetative cells (~10^7 cells) into either 450 µL of defibrinated horse blood (Lampire Biological Labs, Pipersville, PA) or 2.5% HIB supplemented with 50% inactivated horse serum (Sigma) and 0.8% sodium bicarbonate and incubating at 37°C for 3 h.

Clinical Specimens

For liquid specimens, ~90 µL of each specimen was diluted with 10 vol of PBST; the cells were recovered by centrifugation (14, 000 X *g* for 3 min). After removal of supernatant, the cells were suspended in 90 µL of the residual supernatant. Solid tissues (e.g., lymph nodes, lung tissues) were homogenized with a small disposable tissue grinder (Kendall Co., Mansfield, MA) in 100–250 µL of HIB. Forty-five microliters of the homogenates or cell suspensions was used directly in the DFA assays.

CW- and CAP-DFA Assays

To evaluate the sensitivity and specificity of both DFA assays, 45-µL cell suspensions were mixed with 5 µL of anti-CW FITC or anti-CAP FITC conjugate and incubated at 37°C for 30 min. After the reaction mixture was diluted with 1 mL PBST, the cells were recovered by centrifugation (14, 000 X *g* for 3 min) and washed once more with 1 mL deionized water. After the second centrifugation, most of the supernatant was aspirated, and the cell pellet was suspended in ~100 µL of the residual water. A 2-µL volume of the suspension was transferred to one well of a 12-well Teflon-coated microscope slide (Cel-Line/Erie Scientific Co., Portmouth, NH), air-dried, and mounted with DAKO faramount aqueous medium (DAKO Co., Carpinteria, CA). The labeled cells were visualized on a UV microscope with a 40X or 100X objective with oil. When *B. anthracis* cells exhibited whole-body bright green fluorescence against a dark background, the reaction was read as positive. A negative reaction had cells that did not show fluorescence. An identical procedure was used to stain 45-µL volumes of the processed clinical specimens. DFA was reported as positive for *B. anthracis* only when both CW- and CAP-DFA assays were positive.

To determine the lower limits of detection for both CW- and CAP-DFA assays, serial 10-fold dilutions (10^7–10^3 cells/mL) of the fresh cells of the control strains (Pasteur strain and *B. cereus*) were prepared, and 45-µL volumes of cell suspension were used as described.

Results

Of 230 *B. anthracis* isolates analyzed, 228 (99%) were positive in the CW-DFA (Table 1) (19). Two isolates (one environmental isolate from a mill and one from a cow) that

were negative by the CW-DFA assay were collected in Alabama in the 1950s (20). Among the non–*B. anthracis* isolates, 10 (43%) of 23 *B. cereus* and 2 (16.7%) of 12 *B. thuringiensis* were also CW-DFA positive. All 9 *B. subtilis,* 11 *B. megaterium,* and 1 *B. mycoides* strains were negative (Table 2). In all the positive reactions, >99% of the *B. anthracis* cells expressed cell wall polysaccharide antigen so that characteristic chain-forming rods were visualized with bright fluorescence (Figure, panel A). All the 149 *B. anthracis* isolates from the 2001 anthrax outbreak investigation were positive (Table 1). The sensitivity and specificity of the CW-DFA assay were 99% (228/230, 95% confidence intervals [CI]) and 78.6% (44/56, 95% CI), respectively.

All but 1 (99.7%) of the 230 B. anthracis isolates tested were CAP-DFA positive; the single exception was a *B. anthracis* Sterne strain cured of plasmid pXO2 (Table 1) and, thus, as expected, it was unencapsulated. Of the 56 non–*B. anthracis* isolates tested, only 1 *B. megaterium* strain was positive by the CAP-DFA assay (Table 2). This environmental isolate, collected during the bioterrorism-associated anthrax outbreak, was identified as *B. megaterium* by both standard microbiologic procedures (7) and sequencing of the 16S ribosomal RNA gene (data not shown). All the 149 *B. anthracis* isolates from the 2001 anthrax outbreak were CAP-DFA positive. Most of the encapsulated *B. anthracis* cells (>90%) were labeled uniformly (Figure, panel B), and they demonstrated similar fluorescence to that of the cell wall staining. The sensitivity and specificity of the CAP-DFA assay were 99% (229/230, 98% to 100% CI) and 98% (55/56, 90% to 100% CI), respectively.

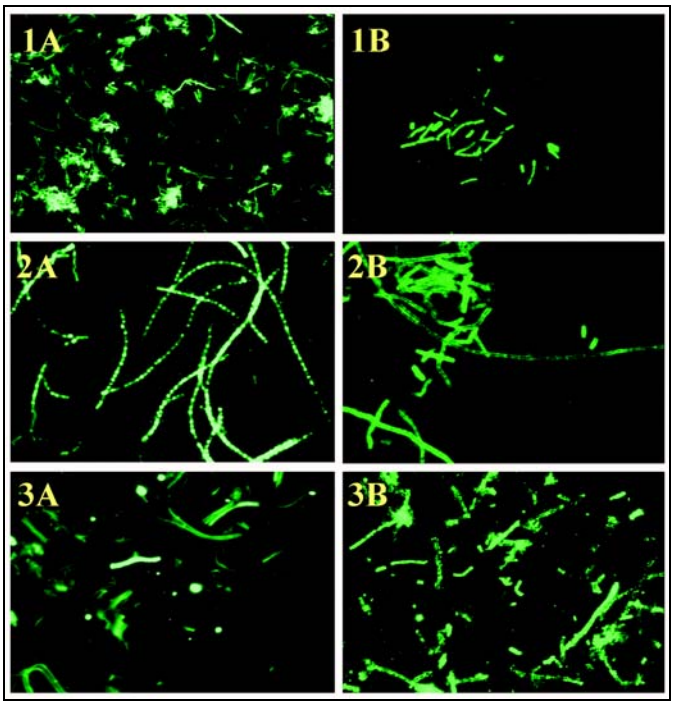

Figure. Direct fluorescent-antibody (DFA) staining of *Bacillus anthracis* cells. Panel A (cell wall DFA) and Panel B (capsule DFA) correspond to 1) Positive control (*B. anthracis* Pasteur strain), 2) Test isolate #2002013601 (environmental specimen, 2001 U.S. anthrax outbreak), and 3) Clinical specimen #2002007069 (lung tissue of patient 1, 2001 U.S. anthrax outbreak), original magnification x 400.

Analysis of the combined DFA assay results showed that 227 of 230 *B. anthracis* isolates were positive, yielding a specificity of 99% (95% CI, 96% to 100%). Similarly, all 56 of the other *Bacillus* strains were negative, for a specificity of 100% (95% CI, 94% to 100%). The current two-component DFA assay was capable of detecting as low as ~10^4 cells/mL of vegetative or encapsulated *B. anthracis* cells from cultures.

Fourteen of the 26 clinical specimens analyzed from seven patients with laboratory-confirmed anthrax were positive in both the CW- and CAP-DFA assays (Table 3). Furthermore, most blood specimens (8 of 12) were positive by both assays. Most blood specimens were also positive by culture (n=5) and polymerase chain reaction (PCR) (n=4) assays. Among the other clinical specimens tested, two lung tissues, one lymph node, two pleural fluids, and one unspecified body fluid were positive by both DFA assays. Four of these six specimens were negative by culture, and three of them were positive by PCR. Most of these specimens were collected from patients treated with antimicrobial agents before or on the day of specimen collection. All other clinical specimens, such as heart fluid, pericardial fluid, and chest fluids, were negative by both DFA assays.

Discussion

Recent events have emphasized the need for rapid, sensitive, and specific assays for the confirmatory identification of *B. anthracis* and detection of this agent directly in clinical specimens. The availability of monoclonal antibodies recognizing the cellwall polysaccharide and capsule antigens of vegetative cells provides the means to rapidly differentiate *B. anthracis* from other *Bacillus* spp. Although some *B. cereus* and *B. thuringiensis* strains express the galactose/N-acetylglucosamine polysaccharide antigen, such organisms lack the poly-γ-D-glutamic acid capsule of *B. anthracis.* Thus, detection of both antigens by a DFA assay is highly specific for *B. anthracis*. In this study, we evaluated a two-component DFA assay employing monoclonal antibodies specific for these two antigens for confirmatory identification of diverse *B. anthracis* strains and for detection of *B. anthracis* directly in clinical specimens. We found that this approach provided sensitive and specific confirmation of *B. anthracis* cultures within 3–6 h. In addition, this approach detected *B. anthracis* directly in clinical specimens of seven patients with laboratory-confirmed inhalational anthrax.

The expression of DFA targets could vary by *B. anthracis* strain, which would adversely affect the sensitivity of the test. Consequently, we first evaluated the sensitivity of the two DFA assays independently against 230 *B. anthracis* isolates. Because of the diversity of *B. anthracis* isolates tested, our results should be applicable to very divergent strains from different sources. The sensitivity for *B. anthracis* was high (99%) for each DFA. The CW-DFA assay failed to detect only two

Table 1. Origin, designations, and results of cell wall and capsule direct fluorescent-antibody assays for 230 *Bacillus anthracis* isolates[a]

Origin	No. of isolates	Temporal range and geographic origin	MLVA genotypes represented[b]	CW-DFA (% positive)	CAP-DFA (% positive)
Human isolates	31	1943–1997; Africa, Asia, Australia, Europe, North America	3,4,22,23,28,32,34,35,36,37,41, 43,44, 45,50,66,68	31 (100)	31 (100)
Animal isolates	29	1939–1997; Africa, Asia, Australia, Europe, North and South America	3,10,20,26,29,30,35,38,40,45,48,49, 51,55,57,78,80,81,84,85,87, 89	29 (100)	29 (100)
Environmental isolates (e.g., soil, burial sites, wool, tannery, mill)	16	1950–1993; Africa, Asia, Europe, and North America	13,14,21,24,47,62,69,73,77,79,82	15 (94)	16 (100)
pX01 plasmid cured	4	1950–1974; North America		3 (75)	4 (100)
pX02 plasmid cured	1	Africa		1 (100)	0 (0)
2001 anthrax outbreak	149	October 2001; United States	62[c]	149 (100)	149 (100)
Total	230			228 (99)	229 (99.6)

[a]DFA, direct fluorescent antibody assay; CW, cell wall; CAP, capsule; MLVA, multiple-locus variable-number tandem repeat analysis.
[b]Keim P, et al. J Bacteriol 2000;182:2928–36 (19).
[c]Hoffmaster et al. Emerg Infect Dis 2002;8:1111-6 (20).

isolates, and the CAP-DFA assay was negative only for the strain cured of the pXO2 plasmid, rendering it unencapsulated. This level of specificity of this two-component DFA assay was affirmed, as every outbreak-associated *B. anthracis* isolate tested positive. We determined that the minimal number of CFU detectable by either assay was 10^4 CFU/mL, a level comparable with that of many PCR assays.

The lower limit of detection is not a limiting parameter of the confirmatory test's sensitivity because unlimited quantities of cells are available for testing after primary culture. However, specificity is crucial; CAP-DFA assay specificity was very high (98%), but the cell-wall assay specificity was only 78.6% compared with the previous studies on the limited cross-reactivity with the other *Bacillus* spp. (12). Almost 93% of the CW-DFA assay false-positive isolates were *B. cereus* or *B. thuringiensis*. Only one *B. megaterium* strain was CAP-DFA positive. However, confirmation of virulent *B. anthracis* strains requires that both assays be positive; compliance with that requirement resulted in 100% specificity because no test isolate except *B. anthracis* was positive in both assays (20). Again, the high specificity of the two-component DFA assay was reflected in its performance on the 149 tested isolates from the 2001 anthrax outbreak. These isolates were shown to be indistinguishable from each other based on the molecular analysis, as delineated by Hoffmaster et al. (20). The DFA assay specificity was similar to the highest levels achieved by PCR assays and the phenotypic confirmatory identification scheme described previously (5,7). However, the two-component DFA assay requires less sophisticated equipment, reagents, and controls and smaller dedicated space than PCR, and is only slightly less rapid. The DFA assay is considerably more rapid than the standard confirmatory identification methods and offers a substantial specificity advantage.

Table 2. Results of cell wall and capsule direct fluorescent-antibody assays for 56 strains of five *Bacillus* species[a]

Species	No. of strains	CW-DFA (% positive)	CAP-DFA (% positive)
B. cereus	23	10 (43)	0 (0)
B. thuringiensis	12	2 (17)	0 (0)
B. megaterium	11	0 (0)	1 (11)
B. mycoides	1	0 (0)	0 (0)
B. subtilis	9	0 (0)	0 (0)
Total	56	12 (21)	1 (1.7)

[a]DFA, direct fluorescent-antibody assay; CW, cell wall; CAP, capsule.

The availability of clinical material from several anthrax patients from the 2001 outbreak provided an additional opportunity to evaluate this two-component DFA. We used the DFA assay to detect *B. anthracis* directly in the limited number of available clinical specimens and compared these results with those from culture and PCR (21). We noted that all DFA-positive specimens reacted with both components of the assay, suggesting that the sensitivities and specificities of the respective assays were similar, as we previously showed for cultures. The two-component assay detected *B. anthracis* in all specimens that were positive by culture and the confirmatory identification regimen. Moreover, four of the five culture-negative specimens that were positive by DFA assay were also positive by PCR. The fifth such specimen (patient 2, unspecified fluid) and two additional specimens (patient 1, lung tissues) that were not cultured were positive only by the DFA assay. Four other specimens from these two patients were PCR positive, suggesting that the discordant DFA assay results were true positives. None of the specimens collected after the patient received antimicrobial therapy were culture positive, but four specimens collected from four patients concurrent with (n=1) or after (n=3) antimicrobial therapy were DFA positive. Together, these results suggest that the DFA assay is specific for *B. anthracis* and that its sensitivity is similar to that of culture or perhaps considerably greater if the patient is receiving antimicrobial agents. Conversely, six PCR-positive specimens were negative by the DFA assay, indicating that the latter may be relatively less sensitive. The two DFA positive/PCR-negative specimens indicate that only performing all available

Table 3. Results of 26 clinical specimens from seven inhalational anthrax patients analyzed by direct fluorescent-antibody assay, culture, and polymerase chain reaction assay[a]

	Specimen			Results		
Patient identifier[b]	Type	Number	Date collected	DFA	Culture	PCR[c]
1	Heart blood[d,e]	1	10/6	(-)	ND	(-)
1	Blood[d,e]	1	10/6	(-)	ND	(-)
1	Lung tissue[d,e]	2	10/6	(+)	ND	(-)
1	Chest fluid[d,e]	2	10/6	(-)	ND	(+)
1	Pericardial fluid[d,e]	1	10/6	(-)	ND	(+)
2	Blood[d]	3	10/5	(-)	(-)	(-)
2	Pleural fluid[d]	1	10/5	(+)	(-)	(+)
2	Pleural fluid[d]	1	10/5	(-)	(-)	(+)
2	Unspecified body fluid[d]	1	10/5	(+)	(-)	(-)
3	Blood[d]	1	10/19	(+)	(-)	(+)
5	Blood[d]	2	10/21	(+)	(+)	(+)
6	Blood	1	10/22	(+)	(+)	(+)
10	Lung tissue[d,e]	1	10/31	(-)	(-)	(+)
10	Lymph node[d,e]	1	10/31	(-)	(-)	(+)
10	Pleural fluid[d]	1	10/29	(+)	(-)	(+)
10	Pleural fluid[d]	1	10/29	(-)	(-)	(+)
11	Blood	2	11/17	(+)	(+)	ND
11	Blood	2	11/17	(+)	(+)	ND
11	Lymph node[d-f]	1	11/21	(+)	(-)	(+)

[a]DFA, direct fluorescent-antibody assay; PCR, polymerase chain reaction; ND, not done.
[b]Patients 1, 2, 3, 5, 6, 10 reported by Jernigan et al. (6), and patient 11 reported by Barakat et al. (18).
[c]Real-time PCR as described by Hoffmaster et al. (20). All DNA samples tested positive by human beta actin PCR.
[d]Specimens collected the day on or after antimicrobial treatment was begun.
[e]Specimens collected postmortem.
[f]Documented culture negative; previously reported as culture positive (18).

assays on specimens may maximize diagnostic sensitivity. The two-component DFA assay rapidly detected *B. anthracis* in all seven anthrax patients, suggesting that its predictive value may have diagnostic relevance. However, the numbers of specimens and patients in this evaluation were limited.

DFA assays have traditionally been used to rapidly identify bacterial cultures and to directly detect bacterial disease agents in infected clinical specimens. The extensive use of such assays depends on their ability to sensitively and specifically detect target organisms and to predict the diseases they cause. We report for the first time an evaluation of a two-component DFA assay to confirm the identity of presumptive *B. anthracis* cultures and to detect this agent in clinical specimens. The current assay had excellent sensitivity and specificity as a rapid confirmatory test for *B. anthracis* cultures performed in a real-time fashion in an outbreak setting. The assay also detected *B. anthracis* in a limited number of specimens from anthrax patients. However, we recommend that this latter application be limited to a presumptive role in the laboratory diagnosis of anthrax, until positive and negative predictive values are better defined by future evaluations in animal models and human populations with high anthrax prevalence or outbreaks.

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References

1. Pile J, Malone J, Eitzen E, Friedlander A. Anthrax as a potential biological warfare agent. Arch Intern Med 1998:158:429–34.
2. Inglesby T, Henderson D, Bartlett J, Ascher M, Eitzen E, Friedlander A, et al. Anthrax as a biological weapon. JAMA 1999:281:1735–45.
3. Christopher G, Cieslak T, Pavlin A, Eitzen E. Biological warfare. A historical perspective. JAMA 1997:278:412–7.
4. Kliemann W, Ruoff K. Bioterrorism: implications for the clinical microbiologist. Clin Microbiol Rev 2001:14:364–81.
5. Khan A, Morse S, Lillibridge S. Public-health preparedness for biological terrorism in the USA. Lancet 2000:356:1179–82.

6. Jernigan J, Stephens D, Ashford D, Omenaca C, Topiel M, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001:7:933–44.
7. Isenberg H. Clinical microbiology procedures handbook. vol. 1 & 2. Washington: American Society for Microbiology Press; 1992.
8. Phillips A, Ezzell J. Identification of *Bacillus anthracis* by polyclonal antibodies against extracted vegetative cell antigens. J Appl Bacteriol 1989:66 419–32.
9. Franek J. Application of fluorescent antibodies for demonstrating *B. anthracis* in the organs of infected animals. Journal of Hygiene, Epidemiology, Microbiology and Immunology 1964:8:111–9.
10. Helgason E, Ølkstad D, Caugant H, Johansen A, Fouet M, Mock M, et al. *Bacillus anthracis, Bacillus cereus,* and *Bacillus thuringiensis*—one species on the basis of genetic evidence. Appl Environ Microbiol 2000:66:2627–30.
11. Ezzell J, Abshire T. Immunological analysis of cell-associated antigens of *Bacillus anthracis.* Infect Immun 1988:56:349–56.
12. Ezzell J, Abshire T, Little S, Lidgerding B, Brown C. Identification of *Bacillus anthracis* by using monoclonal antibody to cell wall galactose-N-acetylglucosamine polysaccharide. J Clin Microbiol 1990:28:223–31.
13. Fouet A, Mesnage S, Tosi-Couture E, Gounon P, Mock M. *Bacillus anthracis* surface: capsule and S-layer. J Appl Microbiol 1999:87:251–5.
14. Etienne-Toumelin I, Sirard J, Duflot E, Mock M, Fouet A. Characterization of the *Bacillus anthracis* S-layer: cloning and sequencing of the structural gene. J Bacteriol 1995:177:614–20.
15. Ezzell J, Welkos S. The capsule of *Bacillus anthracis*, a review. J Appl Microbiol 1999:87:250.
16. Ezzell J, Abshire T. Encapsulation of *Bacillus anthracis* spores and spore identification. Proceedings of the International Workshop on Anthrax. Salisbury Medical Bulletin 1999:87:42.
17. Mina B, Dym J, Kuepper F, Tso R, Arrastia C, Kaplounova I, et al. Fatal inhalational anthrax with unknown source of exposure in a 61-year-old woman in New York City. JAMA 2002:287:858–62.
18. Barakat L, Quentzel H, Jernigan J, Kirschke D, Griffith K, Spear S, et al. Fatal inhalational anthrax in a 94-year-old Connecticut women. JAMA 2002:287:863–8.
19. Keim P, Price L, Klevytska A, Smith K, Schupp J, Okinaka R, et al. Multi-locus variable-number tandem repeat analysis reveals genetic relationships with *Bacillus anthracis*. J Bacteriol 2000;182:2928–36.
20. Hoffmaster A, Fitzgerald C, Ribot E, Mayer L, Popovic T. Molecular subtyping of Bacillus anthracis and the 2001 bioterrorism-associated anthrax outbreak, United States. Emerg Infect Dis 2002;8:1111-6.
21. Hoffmaster A, Meyer R, Bowen M, Marston C, Weyant R, Barnett GA, et al. Evaluation and validation of a real-time polymerase chain reaction assay for rapid identification of *Bacillus anthracis*. Emerg Infect Dis 2002;8;1178-82.

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Inhalational Anthrax Outbreak among Postal Workers, Washington, D.C., 2001

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In October 2001, four cases of inhalational anthrax occurred in workers in a Washington, D.C., mail facility that processed envelopes containing *Bacillus anthracis* spores. We reviewed the envelopes' paths and obtained exposure histories and nasal swab cultures from postal workers. Environmental sampling was performed. A sample of employees was assessed for antibody concentrations to *B. anthracis* protective antigen. Case-patients worked on nonoverlapping shifts throughout the facility, suggesting multiple aerosolization events. Environmental sampling showed diffuse contamination of the facility. Potential workplace exposures were similar for the case-patients and the sample of workers. All nasal swab cultures and serum antibody tests were negative. Available tools could not identify subgroups of employees at higher risk for exposure or disease. Prophylaxis was necessary for all employees. To protect postal workers against bioterrorism, measures to reduce the risk of occupational exposure are necessary.

In October 2001, four cases of inhalational anthrax occurred in employees at the Washington, D.C., Postal Processing and Distribution Center (DCPDC) (1,2). These cases were part of a multistate outbreak of inhalational and cutaneous anthrax associated with intentional distribution of envelopes containing *Bacillus anthracis* spores to media and federal government offices (2–4). Together, these represent the first reported cases of inhalational anthrax in postal workers and the first reported outbreak of inhalational anthrax caused by occupational exposure in the United States since 1957 (5,6).

The investigation and public health response to this outbreak of inhalational anthrax are reported here. The urgent public health response was directed at preventing new cases of inhalational anthrax through the use of prophylactic antimicrobial drugs for persons potentially exposed to *B. anthracis* spores. The public health response also provided useful information about occupational exposure to aerosolized spores in this type of workplace and the performance of potential tools for determining exposure, such as work history, nasal swabs, immune response markers, and environmental sampling.

Methods

Setting and Background

On October 15, 2001, in an office of the Washington, D.C., U. S. Capitol complex, an envelope addressed to Senator Tom Daschle, intentionally contaminated with *B. anthracis* spores, was opened. This event occurred 2 weeks after a report from Florida of the first-ever inhalational anthrax cases related to envelopes containing *B. anthracis* spores; those cases occurred in employees of a media company (3). The Washington, D.C., Department of Health (DCDOH), Office of the Attending Physician, U.S. Capitol, and the Centers for Disease Control and Prevention (CDC) immediately initiated a multiagency public health response and epidemiologic investigation (7). Enhanced surveillance activities for inhalational anthrax in the national Capitol area were established through a cooperative effort of the DCDOH, Virginia Department of Health, Maryland Department of Health and Mental Hygiene, and CDC.

The epidemiologic investigation determined that the *B. anthracis*–contaminated envelope addressed to Sen. Daschle was processed on October 12 at the DCPDC before entering the Capitol mail distribution system. Late on October 19, a DCPDC employee was admitted to a Virginia hospital with a diagnosis of suspected inhalational anthrax. The CDC team visited the DCPDC on October 20. The suspected case-patient worked in an area of the DCPDC where the envelope had not been processed; he also worked in a second mail facility in

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Maryland. The diagnosis of inhalational anthrax was confirmed on October 21 (1,2). The DCPDC and the second mail facility in Maryland were closed on October 21. On October 20–22, three additional cases of suspected inhalational anthrax were identified in the DCPDC employees; two of these patients died (Table 1). *B. anthracis* grew from blood cultures from all patients within 24 hours. *B. anthracis* was confirmed by *B. anthracis*–specific polymerase chain reaction assay at CDC (2).

A second envelope with *B. anthracis* spores, addressed to Sen. Patrick Leahy, was identified on November 16. This envelope was recovered from a sealed drum containing U.S. Capitol mail quarantined on October 17, 2001.

Postexposure Prophylaxis

After confirmation of the first case of inhalational anthrax in a DCPDC employee, antimicrobial postexposure prophylaxis (PEP) was recommended to all DCPDC employees and visitors to the nonpublic mail-processing area (3,8). DCPDC employees who had been absent from work >24 hours in the past 7 days were contacted to identify any additional cases and inform workers of the recommendation for PEP. Beginning October 21, workers were given a 10-day supply of antimicrobial therapy, pending further investigation. DCPDC employees returned to the public health department antimicrobial agent distribution centers to receive an additional 50-day supply of antimicrobial therapy. All DCPDC employees were offered free medication from the U.S. National Pharmaceutical Stockpile at D.C. General Hospital through a cooperative effort of the DCDOH and the U.S. Public Health Service. Employees could choose to obtain appropriate medication from other sources. The United States Postal Service (USPS) notified employees from the DCPDC about the recommendation for postexposure prophylaxis and urged them to comply. Information on the symptoms of inhalational anthrax, the biology of *B. anthracis*, and possible adverse effects from antimicrobial agents was distributed to postal workers. The number of employees who obtained antimicrobial therapy from D.C. General Hospital, the Virginia Department of Health, and Maryland Department of Health and Mental Hygiene was recorded.

Table 1. Characteristics of inhalational anthrax cases among employees of the Washington, D.C., Processing and Distribution Center[a]

	Case 1	Case 2	Case 3	Case 4
Age (yrs)	56	56	55	47
Race	AA	AA	AA	AA
Date symptoms began	10/16	10/16	10/16	10/16
Date of suspected IA diagnosis	10/19	10/20	10/21	10/22
Date IA confirmed	10/21	10/22	10/23	10/26
Underlying medical conditions	No	No	Yes[b]	Yes[c]
Death due to IA (date)	No	No	Yes (10/21)	Yes (10/22)

[a]AA = African-American, IA=inhalational anthrax.
[b]Diabetes mellitus, sarcoidosis.
[c]Asthma.

Postal System Assessment

In collaboration with the USPS and the Postal Inspection Service, we assessed routine mail-handling procedures and reviewed the path of the two envelopes that were known to contain *B. anthracis* spores. From unique envelope markings, postal inspectors determined the time of automated envelope processing and the machinery used during processing. To establish the number of employees potentially exposed during the passage and processing of the two envelopes, DCPDC employee work zone locations, job descriptions, and assigned work shifts were obtained from USPS administrative data.

Case Exposure Histories

We interviewed surviving case-patients and close associates of those who died by using a standard exposure questionnaire. Case-patients were assessed for job description, work and break locations, travel and medical history, and potential exposure to natural reservoirs of *B. anthracis* spores. Timecard logs established exact times of work during October 11–21.

Environmental Assessment

Beginning October 23, the DCPDC facility was sampled extensively with a combination of surface wipes, surface vacuum samples, and air vacuum samples, reported in detail elsewhere (9–11).

Nasal Swab Cultures

Nasal swab cultures from the DCPDC employees and those who reported visiting that facility during the period October 10–21 were obtained on October 21–22 during the distribution of antimicrobial therapy. Specimens were processed by standard microbiologic methods at the Maryland Department of Health laboratory (12).

Serologic and Exposure Survey

We conducted a survey to evaluate occupational exposures of workers and determine whether there was evidence of immunologic response to *B. anthracis* protective antigen. Exposure histories and serum samples were obtained from a convenience sample of DCPDC employees who went to D.C General Hospital on October 29–30 for their additional 50-day supply of antimicrobial therapy. Each participant was asked to allow a serum sample to be collected and to be individually interviewed with the standardized exposure questionnaire used for case-patients. Informed consent was obtained from all participants.

One blood sample was obtained from each participant. The serum was separated and stored at 4°C. Anti-protective antigen immunoglobulin G (anti-PA IgG) antibody concentrations in

serum specimens were determined by a quantitative enzyme-linked immunoassay described in detail elsewhere (13).

Comparison of Case-Patients and Survey Participants

We compared exposure histories and underlying diseases of the case-patients with the sample of surveyed workers to clarify factors that may have contributed to the four cases of inhalational anthrax at DCPDC. Data from the standardized exposure questionnaire from the DCPDC cases and the other sampled employees were compared by a case-control analysis with two-tailed Fisher exact tests for dichotomous variables or the Wilcoxon signed-ranks test for continuous variables; p values of <0.05 were considered significant.

Results

Postexposure Prophylaxis

Of 2,403 employees at the DCPDC, 1,870 (78%) were recorded as receiving a 50-day supply of antimicrobial therapy at DCDOH, Virginia Department of Health, or Maryland Department of Health and Mental Hygiene postexposure prophylaxis distribution centers. Five members of the CDC team received PEP.

Postal System Assessment

The DCPDC is a 500,000-square foot facility (Figure 1). Approximately 59 million pieces of incoming mail were processed at the DCPDC during October 11–21. The two contaminated envelopes entered the DCPDC on the evening of October 11 or early morning of October 12 in a tray of envelopes originating at the processing and distribution center in Trenton, New Jersey. This tray was taken from the dock (Figure 1, point A) to a large tray-sorting machine (Figure 1, point B) and then moved to a high-speed envelope-sorting machine known as a delivery bar-code sorter (DBCS). The DBCS moves up to 30,000 letters per hour into a series of bins for subsequent distribution. At the end of each work shift, the DBCS is cleaned by a procedure that blows compressed air (70 lbs per square in) into the machine. Unique processing markings on the two envelopes showed that DBCS number 17 (Figure 1, point C) sorted both envelopes on October 12 between 7:05 and 7:30 a.m. The two letters appeared to be processed within minutes of each other.

The envelope addressed to Sen. Daschle was sorted into a bin destined for the U.S. Capitol, taken out of the DBCS number 17, and moved to the government mail section (Figure 1, point D). The government mail section handles distribution of all letters to U.S. government addresses in the metropolitan D.C. area. Routinely, in a process known as riffling, envelopes are flipped through individually for manual confirmation of appropriate sorting. The envelope addressed to Sen. Daschle transited the government mail section on October 12 between 7:30 a.m. and noon, at which time it was dispatched from the loading dock (Figure 1, point E) to the U.S. Capitol's mail distribution facility.

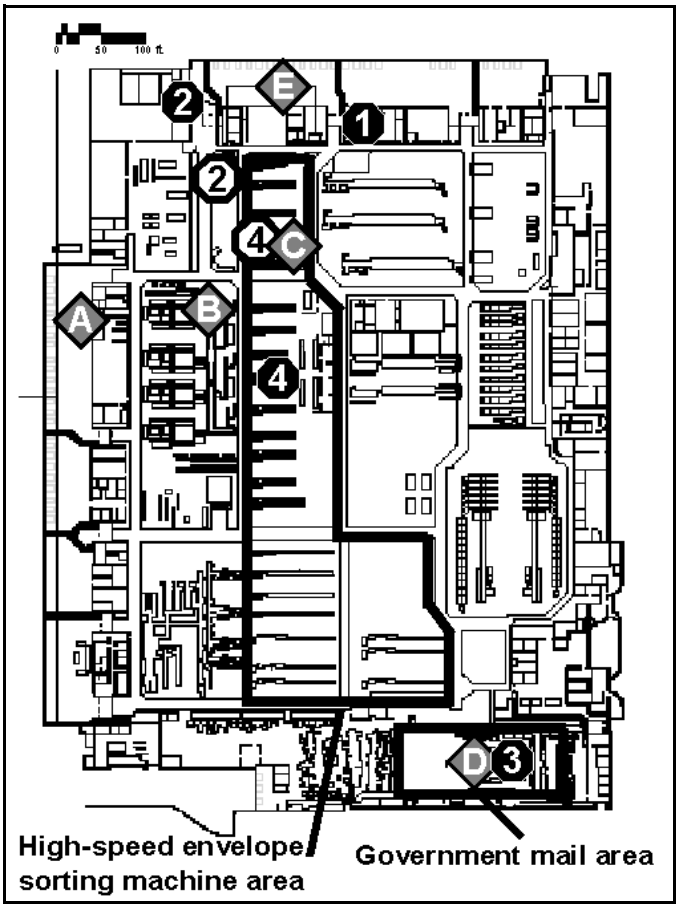

Figure 1. Floor map of the Washington, D.C., Postal Processing and Distribution Center with the known locations of the two *Bacillus anthracis*–contaminated envelopes (gray diamonds with letters) and work locations of case-patients (black circles with numbers) in the facility on October 12, 2001. The estimated location of case-patients during the time of processing the contaminated envelopes at point C, when the letters were processed by the high-speed sorter machine, are shown as open circles. The main processing area of the facility, containing all of the high-speed sorter machines, and the government mail section of the facility are marked.

The envelope addressed to Sen. Leahy was incorrectly sorted as destined for the U.S. State Department, State Annex 32, which has an independent small mail-processing facility in Virginia. The exact path of this envelope is unclear from October 12 to 17. On October 17, the Federal Bureau of Investigation quarantined all remaining U.S. Capitol mail and placed it into sealed drums for further investigation; the Leahy envelope was found in one of these drums on November 16. The letter appeared to be leaking. Routine procedures for redirecting incorrectly sorted envelopes destined for a U.S. government address usually involve employees in the DCPDC government mail section. If routine procedures had been followed, and the envelope were recognized as incorrectly sorted, the envelope would have been manually redirected in the DCPDC government mail section in the period October 12–16.

A fifth case of suspected inhalational anthrax in a postal worker in Virginia was reported on October 25, 2001. The case-patient worked in State Annex 32 (3). Whether the envelope to Sen. Leahy remained in the DCPDC or transited

through the State Annex 32 is not known. Mail destined for the State Annex 32 was sorted at the same time in DBCS number 17 as the envelope addressed to Sen. Leahy. Environmental sampling results from State Annex 32 showed widespread contamination with *B. anthracis* spores, similar to the DCPDC.

Of the 1,961 employees of the DCPDC nonpublic mailroom area, 610 (31%) were assigned to work in one of the same work zones as the four case-patients from this facility. During the time of the two envelopes' passage and processing through the DCPDC, approximately 108 (6%) worked in the main processing area with the DBCS machines between 12 a.m.–12 p.m. on October 12, and 87 (4%) employees worked in the government mail section (Figure 1). Two of the four case-patients worked in one of these work zones. The attack rate for inhalational anthrax in these combined areas was 1% (2/195).

Exposure Histories

The clinical characteristics of the case-patients have been described.(2) All four case-patients from the DCPDC were African-American; case-patient 5 from the mail-processing facility for the State Department was white (Table 1). Only two case-patients had underlying medical conditions. Case-patient 3 had adult-onset diabetes mellitus and a 30-year history of sarcoidosis, although the patient was not on medication for either condition (14). Case-patient 4 had a diagnosis of asthma and was periodically treated with bronchodilators.

Two of the four case-patients from DCPDC worked within several meters of the path of the processed envelopes (Figures 1 and 2). Only one case-patient routinely worked directly with high-speed envelope-sorting machinery, including routine overtime on DBCS number 17. At the time the two contaminated envelopes were sorted in the DCPDC by DBCS number 17, only case-patients 2 and 4 were physically in the DCPDC facility (Figure 2). However, case-patient 1 returned during the window of time when DBCS number 17 was cleaned by blowing compressed air into the machine, between 8:00 a.m. and 9:40 a.m. Case-patient 3 returned to work in the government mail section (Figure 1, point D) at 8:00 p.m. on October 12.

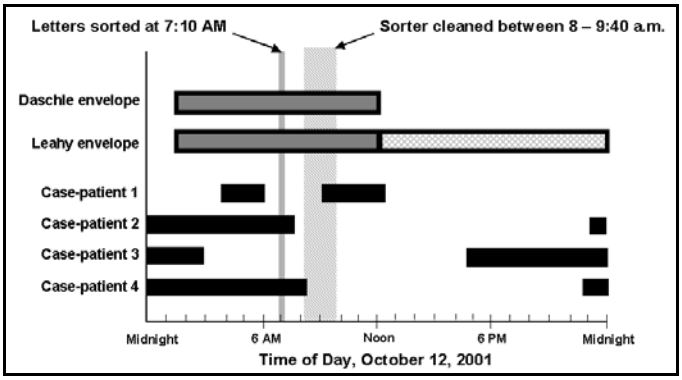

Figure 2. Comparing the time period that the case-patients were at the Washington, D.C., Postal Processing and Distribution Center (solid black bars) to the time period that the two envelopes containing *Bacillus anthracis* spores were processed at the facility (gray bars = known location, gray hatched bars = unknown location) on October 12, 2001. The time that the high-speed sorting machine (delivery bar-code sort number 17) was cleaned, by blowing compressed air into the machine, is denoted by the gray striped area.

Environmental Sampling

Diffuse environmental contamination with *B. anthracis* was found throughout the nonpublic mail-processing area of the DCPDC, particularly on DBCS number 17 and in the government mail section of the facility (9–11). In addition, two supply air ventilation diffusers, located above the area where two of the case-patients worked, were contaminated with *B. anthracis* spores (10,11). None of the samples taken from the public area of the facility were positive for *B. anthracis* spores.

Nasal Swab Cultures

Nasal swab cultures from 3,110 DCPDC employees and visitors, collected 9–10 days after the two envelopes were processed at the DCPDC, were negative for growth of *B. anthracis*.

Seroprevalence and Exposure Survey

On October 29–30, a total of 1,657 employees and visitors to the DCPDC went to D.C. General Hospital to receive additional antibiotic supplies. Of these, 784 (47%) were asked to participate in the survey; 224 (29%) of 784 DCPDC employees participated. Serum samples were obtained from 202 (94%). None of the 202 serum samples had significant detectable specific IgG antibody concentrations of anti-PA IgG, including the three participants who reported a remote history of anthrax vaccination.

The routine work activities of case-patients were also relatively common for the surveyed DCPDC workers (Table 2). Fifty-four percent reported that they manually sorted mail, and 39% reported that they riffled mail. Seven percent of survey participants reported that they riffled mail on October 12. Few employees reported the use of masks (7%), although 47% of survey participants and 50% of case-patients reported using gloves.

Comparison of Case-Patients and Survey Participants

Differences in underlying medical conditions or workplace exposures between the DCPDC case-patients and the survey participants were not statistically significant (Table 2). With sarcoidosis included as a chronic lung disease, more case-patients had chronic lung disease than did survey participants (50% vs. 9%; odds ratio 9.65; 95% confidence interval 1.29 to 72.2; p=0.01). None of the case-patients currently smoked cigarettes, compared with 24% of the participants. Specific mail-handling activities such as manually sorting mail or working on a sorting machine also did not differ. No case-patients and few (10%) of the serosurvey participants handled bulk mail.

Discussion

At least two letters containing *B. anthracis* spores were processed at the DCPDC facility on October 12, 2001, resulting in an outbreak of four cases of inhalational anthrax in

Table 2. Comparison of characteristics and potential exposures among case-patients and survey participants from the Washington, D.C., Processing and Distribution Center

Characteristics and potential exposures	Cases (n=4) N (%)	Participants (n=214) N (%)	OR (95% CI)[a]	p value
Characteristics				
Median years of age (range)	56 (47–56)	49 (25–71)		0.31
Underlying medical conditions				
Any underlying condition	2 (50)	51 (24)	3.2 (0.44 to 23.3)	0.23
Chronic lung disease[b]	1 (25)	20 (9)	3.6 (0.38 to 38.8)	0.25
Diabetes mellitus	1 (25)	18 (8)	3.6 (0.38 to 38.8)	0.25
Heart disease	0	15 (7)		0.58
Liver disease	0	4 (2)		0.78
Recent corticosteroid use[c]	0	9 (4)		0.78
Potential exposures				
Manually sorted mail	3 (75)	115 (54)	2.6 (0.23 to 64.8)	0.63
Riffled mail	3 (75)	84 (39)	4.6 (0.4 to 116.9)	0.30
Worked on sorter machine	1 (25)	75 (35)	0.61 (0.02 to 6.8)	1.00
Bacillus anthracis vaccination	0	3 (1)		1.00
Worked on 10/12/2001	4 (100)	178 (83)		0.48

[a]OR, odds ratio. CI, confidence interval.
[b]Chronic lung disease includes asthma, bronchitis, emphysema, and obstructive lung disease.
[c]Use within previous 2 weeks.

postal employees who worked in that facility. Our investigation demonstrated widespread contamination of the facility with *B. anthracis* spores, including areas through which the two letters were unlikely to have traveled. The case-patients did not all work directly along the path of the contaminated envelopes as they were processed through the facility on October 12, and two patients were not even in the building at the time of mechanical sorting. Therefore, inhalational anthrax likely resulted from multiple aerosolization events, including processing of the letters through the high-speed sorting machine, manual sorting and riffling of mail, and cleaning the high-speed sorting machine by blowing compressed air into it. Evaluation of re-aerosolization of *B. anthracis* spores at the DCPDC, conducted after partial cleaning of the high-speed sorter that processed the *B. anthracis*–containing envelopes, DBCS number 17, identified ongoing low-level aerosolization after the machine was turned on, suggesting aerosolized spores were likely present at some level throughout the 10 days from October 12 until the facility closed on October 21 (15).

Before recognition of inhalational anthrax among postal workers in Washington, D.C., and New Jersey, two cases of inhalational anthrax and several cases of cutaneous anthrax were identified in Florida and New York in employees of media companies; the latter cases were associated with contaminated envelopes postmarked at the Trenton Processing and Distribution Center (PDC) September 18, 2001 (2,7). Despite this, the first recognition of inhalational disease in the postal service occurred in Washington, D.C., associated with letters processed in the Trenton PDC, October 9, 2001. Why the envelopes processed in October resulted in cases of inhalational anthrax among postal workers while those processed in September did not is unclear. A likely possibility is that the characteristics of the *B. anthracis* preparation or the condition of the envelope(s) at the time of transits through the DCPDC in October (or both) differed from that in September (16). The events that occurred in October in Washington, D.C., suggest the need to ensure that future bioterrorism events involving *B. anthracis* contamination of envelopes incorporate new understanding of the aerosolization potential in the PDC environment, the need for extensive traceback of contaminated envelopes, and broad initiation of prophylaxis to all persons potentially exposed to spores.

Given the widespread contamination of the DCPDC and the likelihood of multiple aerosolization events, why the four case-patients developed inhalational anthrax but other workers in the same facility did not is not clear. Some underlying medical conditions may make persons more susceptible to inhalational anthrax during the initial period after exposure, although we were unable to demonstrate this conclusively in this investigation, primarily because of small numbers. Many employees in the DCPDC performed activities at work that might have resulted in aerosolization of spores. Given the potential for a long incubation period, especially after low-dose exposures (17,18), and documented re-aerosolization (15), many additional cases of inhalational anthrax were likely prevented by the postexposure prophylaxis given to all facility employees 9 days after the two envelopes were processed at the DCPDC.

More than 2,000 postal employees were advised to take 60 days of antimicrobial agents to prevent inhalational anthrax.

We used currently available methods, including nasal swab cultures, a serologic assay, and environmental sampling, to identify DCPDC workers who were exposed to *B. anthracis* spores. While the environmental sampling and exposure survey suggested that many persons could have been exposed to *B. anthracis*, neither the nasal swab cultures nor serologic survey could reliably identify subgroups of DCPDC workers who were exposed and thus at higher risk of developing inhalational anthrax. Therefore, among DCPDC employees, postexposure prophylaxis was necessary for all workers in the facility. Until better methods to determine exposure to *B. anthracis* and to assess risk factors for development of inhalational anthrax are available, broad implementation of postexposure prophylaxis to all persons potentially exposed will be necessary. Vaccines may play a role in postexposure prophylaxis, in addition to their recognized role in preexposure prophylaxis for persons from selected high-risk occupations.

Serologic analyses for *B. anthracis* have been developed to confirm seroconversion after anthrax vaccine administration (19) but have been used to provide retrospective confirmation of cutaneous *B. anthracis* infection (20,21). During this bioterrorism event, the anti-PA IgG antibody assay was developed, validated, and used to confirm clinical cases of disease for the first time; the assay had good sensitivity and specificity to detect clinical disease (13). As demonstrated here, this IgG assay was not able to determine whether persons without clinical disease were exposed or infected with *B. anthracis*; whether an anti-PA IgM antibody assay would improve sensitivity is unknown. Although we did not obtain serum specimens from DCPDC employees at a longer interval after exposure, an investigation of employees on Capitol Hill failed to detect anti-PA IgG antibody as late as 6 weeks after exposure (7). Additionally, all DCPDC participants in the survey had been taking antimicrobial agents since October 21–22; the antimicrobial agents may have blunted the immune response. Nasal swab cultures collected 9 days after the two envelopes were processed at the DCPDC were also negative. These findings may be due to many factors, including low exposure to spores, the transient nature of *B. anthracis* spores in the nasal passages, or the low sensitivity of this assay. Previous studies have isolated *B. anthracis* in nasal passages long periods after exposure (4,6); however, the characteristics of the spores disseminated throughout the DCPDC may not be similar to those previously studied. Environmental sampling detected *B. anthracis* spores in the DCPDC but at this time cannot determine the inoculum size. In addition, the correlation between environmental culture data and risk for disease remains unclear. In light of these limitations, multiple criteria, including epidemiologic and environmental results, should be considered when deciding whether prolonged postexposure prophylaxis is warranted.

Because of the unprecedented nature of this outbreak, the risks of inhalational anthrax associated with exposure to *B. anthracis* spores were unknown when we began our investigation. We have learned that the preparations of *B. anthracis* spores used in this event had a high potential for diffuse aerosolization, especially in settings such as the DCPDC. Our current diagnostic tools are limited in their ability to identify persons who were exposed to spores and likely to become ill; future studies are needed to improve these tools. In spite of this, many procedures that increased the likelihood of spore dissemination in PDC facilities have been identified and can be modified to reduce the risk to workers in the future. For example, the practice of blowing compressed air into sorting machines has been discontinued, and use of appropriate respiratory protective equipment could be encouraged (22). Occupational safety of postal workers from bioterrorism and other health hazards can be enhanced with attention to engineering, procedural safety measures, and personal protective equipment. The public health response to future bioterrorism events that involve *B. anthracis* spores should include extensive traceback of contaminated envelopes and broad use of prophylactic measures to prevent disease.

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References

1. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
2. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
3. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
4. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–93.
5. Plotkin SA, Brachman PS, Utell M, Bumford FH, Atchison MM. An epidemic of inhalation anthrax, the first in the twentieth century: I. Clinical features. 1960. Am J Med 2002;112:4–12.
6. Brachman PS, Plotkin SA, Bumford FH, Atchison MM. An epidemic of inhalation anthrax: the first in the twentieth century. II. Epidemiology. Am J Hyg 1960;72:6–23.
7. Hsu VP, Lukacs SL, Handzel T, Hayslett J, Harper S, Hales T, et al. Opening a Bacillus anthracis-containing envelope, Capitol Hill, Washington, D.C.: the public health response. Emerg Infect Dis 2002;8;1039-43.

8. Bell DM, Kozarsky PE, Stephens DS. Clinical issues in the prophylaxis, diagnosis, and treatment of anthrax. Emerg Infect Dis 2002;8:222–5.
9. Centers for Disease Control and Prevention. Evaluation of *Bacillus anthracis* contamination inside the Brentwood Mail Processing and Distribution Center—District of Columbia, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:1129–33.
10. Sanderson W, Stoddard R, Echt A, McCleery RE, Picitelli CA, Kim D, et al. Evaluation of *Bacillus anthracis* contamination inside the Brentwood Post Office, Washington, D.C. Report to U.S. Postal Service. Cincinnati: National Institute for Occupational Safety and Health; 2001.
11. Sanderson W, Hein M, Taylor L, Curwin B, Kinnes G, Hales T, et al. Second evaluation of *Bacillus anthracis* contamination inside the Brentwood Mail Processing and Distribution Center, District of Columbia. Report to the U.S. Postal Service. Cincinnati: National Institute for Occupational Safety and Health; 2002.
12. Murray PR. Manual of clinical microbiology. 7th edition. Washington: ASM Press; 1999.
13. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. Emerg Infect Dis 2002;8;1103-10.
14. Brachman PS, Pagana JS, Albrink WS. Two cases of fatal inhalation anthrax, one associated with sarcoidosis. N Engl J Med 1961;265:203–8.
15. Dull PM, Wilson KE, Kournikakis B, Whitney EAS, Boulet CA, Ho JYW, et al. Bacillus anthracis aerosolization associated with a contaminated mail sorting machine. Emerg Infect Dis 2002;8;1044-7.
16. Broad WJ, Johnston D. Anthrax sent through mail gained potency by the letter. New York Times 2002 May 7; Sect. A:1.
17. Meselson M, Guillemin J, Hugh-Jones M, Langmuir A, Popova I, Shelokov A, et al. The Sverdlovsk anthrax outbreak of 1979. Science 1994;266:1202–8.
18. Henderson DW, Peacock S, Belton FC. Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. J Hyg 1956;54:28–36.
19. Iacono-Connors LC, Novak J, Rossi C, Mangiafico J, Ksiazek T. Enzyme-linked immunosorbent assay using a recombinant baculovirus-expressed *Bacillus anthracis* protective antigen (PA): measurement of human anti-PA antibodies. Clin Diagn Lab Immunol 1994;1:78–82.
20. Harrison LH, Ezzell JW, Abshire TG, Kidd S, Kaufmann AF. Evaluation of serologic tests for diagnosis of anthrax after an outbreak of cutaneous anthrax in Paraguay. J Infect Dis 1989;160:706–10.
21. Sirisanthana T, Navachareon N, Tharavichitkul P, Sirisanthana V, Brown AE. Outbreak of oral-oropharyngeal anthrax: an unusual manifestation of human infection with *Bacillus anthracis*. Am J Trop Med Hyg 1984;33:144–50.
22. Centers for Disease Control and Prevention. CDC interim recommendations for protecting workers from exposure to *Bacillus anthracis* in work sites where mail is handled or processed. Health Alert Network [online] Oct. 31, 2001 [cited 2002 May 17]; Available from: URL: http://www.cdc.gov/niosh/unp-mailrecs1.html

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Surveillance for Anthrax Cases Associated with Contaminated Letters, New Jersey, Delaware, and Pennsylvania, 2001

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In October 2001, two inhalational anthrax and four cutaneous anthrax cases, resulting from the processing of *Bacillus anthracis*–containing envelopes at a New Jersey mail facility, were identified. Subsequently, we initiated stimulated passive hospital-based and enhanced passive surveillance for anthrax-compatible syndromes. From October 24 to December 17, 2001, hospitals reported 240,160 visits and 7,109 intensive-care unit admissions in the surveillance area (population 6.7 million persons). Following a change of reporting criteria on November 8, the average of possible inhalational anthrax reports decreased 83% from 18 to 3 per day; the proportion of reports requiring follow-up increased from 37% (105/286) to 41% (47/116). Clinical follow-up was conducted on 214 of 464 possible inhalational anthrax patients and 98 possible cutaneous anthrax patients; 49 had additional laboratory testing. No additional cases were identified. To verify the limited scope of the outbreak, surveillance was essential, though labor-intensive. The flexibility of the system allowed interim evaluation, thus improving surveillance efficiency.

In the fall of 2001, a multistate investigation involving local, state, and federal public health and law enforcement authorities identified letters intentionally contaminated with *Bacillus anthracis* spores; these letters were processed through the Trenton Processing and Distribution Center on September 18 and October 9. On October 13, the New Jersey Department of Health and Senior Services (NJDHSS) received reports of two postal employees with clinical symptoms compatible with cutaneous anthrax; their illnesses began on September 26 and 28. On October 18, following the confirmation of the first anthrax case in New Jersey, the Trenton Processing and Distribution Center was closed. Subsequently, NJDHSS identified a total of six anthrax cases (two inhalational, four cutaneous), all reported from October 13 to 24 (1,2).

On October 24, NJDHSS and the Centers for Disease Control and Prevention (CDC) began formal surveillance for specified clinical syndromes compatible with anthrax. Surveillance was implemented with the objectives of improving case finding, describing the spectrum of clinical signs and symptoms of possible anthrax illness, characterizing the population at risk, and determining the magnitude of the outbreak. This report describes the surveillance efforts and results.

Methods

From October 24 to December 17, NJDHSS and CDC implemented passive surveillance (3) for syndromes compatible with anthrax, supported with specific laboratory testing for *B. anthracis*. This surveillance included two components: stimulated passive hospital-based surveillance (4) for inhalational anthrax and enhanced passive surveillance for inhalational anthrax and cutaneous anthrax.

Stimulated Passive Hospital-Based Surveillance for Inhalational Anthrax

We implemented stimulated passive hospital-based surveillance in 15 counties in New Jersey, Delaware, and Pennsylvania, on October 24. Infection control professionals (ICPs) of all acute-care hospitals of 10 New Jersey, 2 Pennsylvania, and 3 Delaware counties were invited to participate; specialty and psychiatric hospitals were not included in surveillance (Figure 1). Reporting criteria for possible inhalational anthrax included any emergency department patient with a diagnosis of respiratory failure or severe respiratory distress or any intensive-care unit (ICU) patient from whom blood, cere-

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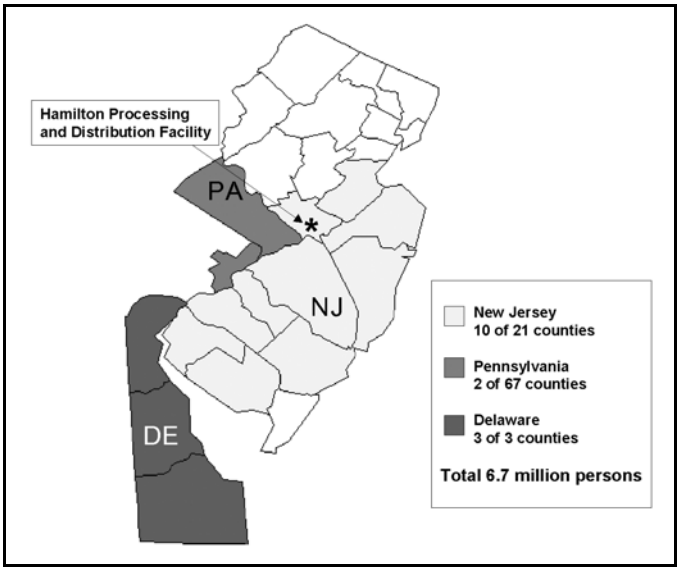

Figure 1. Counties participating in active surveillance, New Jersey, Pennsylvania, and Delaware, 2001.

brospinal fluid, or pleural fluid cultures were obtained. Reporting criteria and forms were distributed to ICPs and local and state health departments by e-mail or fax. We requested that ICPs provide a daily summary report that documented the total number of emergency department visits and ICU admissions that met reporting criteria for possible inhalational anthrax. For each patient whose illness met reporting criteria for inhalational anthrax, ICPs completed a case ascertainment form that provided details on the patient's demographic information and clinical symptoms.

We requested that hospitals provide daily summary reports within 1 day of the date of the reported data. Infection control professionals faxed or e-mailed completed summary and case ascertainment reports to officials in appropriate local and state health departments; these reports were then forwarded to the CDC New Jersey Operations Center at NJDHSS for review. Data summaries were provided periodically to ICPs during the surveillance period.

To improve the surveillance system, we performed interim evaluations and conducted periodic conference calls with local and state health departments and participating ICPs to gain feedback on surveillance methodology. In response to comments that initial inhalational anthrax reporting criteria encompassed a broad spectrum of differential diagnoses including illnesses unlikely to be undiagnosed anthrax, such as chronic pulmonary disease, we modified clinical criteria for reporting on November 8. ICPs were then requested to report any emergency department or ICU patient with illness onset after September 18 with 1) fever, cough, abnormal chest x-ray, and no prior chronic pulmonary disease, 2) fever, respiratory failure, or severe respiratory distress not clearly attributable to a previously diagnosed chronic pulmonary or cardiac disease, or 3) sepsis of unknown origin. We distributed revised reporting criteria to ICPs and local and state health departments through e-mail, facsimile, and telephone communication.

Passive Surveillance for Inhalational or Cutaneous Anthrax

Passive surveillance for possible inhalational anthrax and cutaneous anthrax cases was conducted statewide in New Jersey. Reporting criteria for possible cutaneous anthrax included persons with a suspicious lesion including an ulcer with surrounding erythema, edema, or vesicles, or a blackened eschar forming 3–7 days after the onset of the skin lesion; an ulcerative or necrotic lesion and a history of possible exposure to anthrax, including employment at a postal facility or handling mail in another setting; or laboratory evidence suggestive of *B. anthracis* infection.

Reporting criteria for both inhalational anthrax and cutaneous anthrax were made available on websites of the Medical Society of New Jersey (available at: http://www.msnj.org/), New Jersey Association of Family Physicians (available at: http://www.njafp.org/), and NJDHSS (available at: http://www.state.nj.us/health/); surveillance information was also distributed through press releases to the media.

Suspicious illnesses were reported to the New Jersey Emergency Operations Center and to the CDC New Jersey Operations Center. Nurses, physicians, and epidemiologists from NJDHSS and CDC fielded general and medical inquiries and reviewed reports.

Clinical Follow-Up

After reviewing all surveillance reports, we followed up on reports of patients considered to be at risk based on clinical symptoms or exposure history (e.g., employment at a postal facility and occupations that involved mail handling) or of patients without clear alternative diagnoses. Through interviews with physicians, nurses, ICPs, hospital laboratory staff, and patients, we obtained additional history on clinical symptoms, exposure, and occupational history and any preliminary hospital laboratory results available by the time of follow-up.

Clinical specimens, including whole blood, sera, pleural fluid, and skin biopsies, were obtained from persons with highly suspicious illness or credible exposure history and cultured at the New Jersey Public Health and Environmental Laboratories. CDC laboratories performed additional tests, including immunohistochemical staining of clinical specimens with *B. anthracis* capsule and cell-wall antibody, *B. anthracis*–specific polymerase chain reaction, and serologic detection of immunoglobulin G to *B. anthracis* protective antigen.

Resources Required for Surveillance

To examine the resources required for surveillance, we documented the number and type of persons and organizations and the time required to collect and analyze surveillance data. We also designed and distributed a survey to describe the resources available to hospitals participating in stimulated passive surveillance and to assess ICPs' experiences with surveillance activities. ICPs faxed completed questionnaires to the NJDHSS for analysis.

Medical Examiner Data Review

Concurrent with the surveillance efforts, the New Jersey State Medical Examiner asked all county medical examiners to retrospectively review all unexplained deaths due to acute respiratory illness back to September 1. In addition, all county medical examiners were instructed to accept for autopsy any unexplained deaths due to acute respiratory illness.

Data Management and Analysis

For each participating hospital, we calculated the ratio between the number of daily reports received and the number of expected reports. The expected number of reports per hospital was the number of days a hospital participated in surveillance, calculated from the date of the first report received to December 17.

We used Access 2000 (Microsoft Corp., Redmond, WA) to maintain data and generate summary reports. Data were analyzed using Epi Info 2000, Epi Map version 2, and SAS version 8.0 (SAS Institute, Inc., Cary, NC).

Results

Stimulated Passive Hospital-Based Surveillance

In the three states affected, all 61 acute-care hospitals from 15 counties conducted stimulated passive surveillance. During the 1st week of surveillance, all 26 hospitals in six counties were incorporated into the surveillance system. By the 4th week, as surveillance expanded into additional counties, all hospitals in these areas were incorporated into the system. Seventy-eight percent to 91% of all participating hospitals provided daily summary reports during 6 of 8 weeks of the surveillance period. Reporting rates were lowest during the 1st and last weeks of the surveillance interval (Figure 2).

During the entire 8-week surveillance period, in New Jersey, participating hospitals provided reports a mean of 89% (range 18% to 100%) of days during which they participated in surveillance; in Delaware, the mean was 86% (range 82% to 91%); and in Pennsylvania, the mean was 74% (range 23% to 94%).

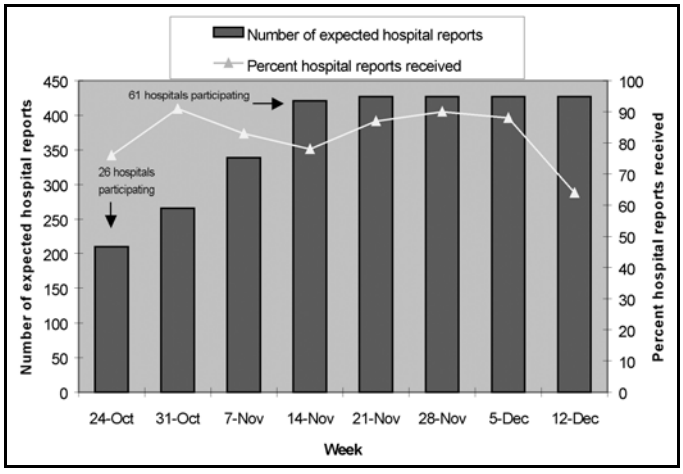

Figure 2. Hospital participation in simulated passive surveillance for possible inhalational anthrax by surveillance week; Delaware, New Jersey, and Pennsylvania; October 24–December 17, 2001.

Thirty-nine (64%) participating hospitals were community-based acute-care facilities; among community-based facilities, participating hospitals provided reports a mean of 88% (range 18% to 100%) of the days during which they participated in surveillance. Twenty-two (36%) participating hospitals were university-based acute-care facilities; participating hospitals provided reports a mean of 84% (range 23% to 100%) of days during which they participated in surveillance.

Following an increase in reporting criteria specificity on November 8, the average of possible inhalational anthrax reports decreased 83% from 18 to 3 per day. The proportion of reports requiring follow-up increased 10% from 37% (105/286) to 41% (47/116).

Reporting of Possible Inhalational Anthrax Illness

During October 24 to December 17, stimulated passive hospital-based surveillance generated reports of 240,160 emergency department visits and 7,109 ICU admissions from a surveillance population of 6.7 million residents. Of these emergency department visits and ICU admissions, 402 patients whose illnesses met clinical criteria for possible inhalational anthrax were identified by ICPs. The clinical investigation team then identified 152 patients whose clinical presentation warranted collection of additional information, of whom 10 (7%) had additional laboratory testing performed at the state or CDC laboratories. Passive surveillance generated a total of 62 reports of patients meeting clinical criteria for inhalational anthrax from over 6,000 phone calls to the New Jersey Emergency Operations Center and the CDC New Jersey Operations Center. After preliminary follow-up of all 62 reports, the CDC or state laboratories performed additional tests on specimens from 13 (21%) of these patients.

No additional inhalational anthrax cases were identified among the 214 reports of possible inhalational anthrax that were followed up. A total of 103 (48%) had follow-up diagnoses of chronic pulmonary or acute infectious processes, including asthma and chronic obstructive pulmonary disease exacerbations (13 reports), bronchitis (6 reports), pneumonia (40 reports), and other pulmonary conditions (44 reports). For the remaining 111 (52%), the diagnosis of anthrax was ruled out but no alternative diagnosis was identified.

Reporting of Possible Cutaneous Anthrax Illness

No new cutaneous anthrax cases were identified among the 98 reports meeting surveillance criteria for cutaneous anthrax, including 26 (27%) that warranted additional testing. Of these 98 reports, 32 (33%) involved follow-up diagnoses of cellulitis (6 reports), herpes zoster (5 reports), contact dermatitis (11 reports), or other dermatologic illnesses, including chronic conditions such as eczema (10 reports). For the remaining 66, the diagnosis of anthrax was ruled out, but no alternative diagnosis was identified.

Resources Required for Surveillance

In the private sector of the population in these states, hundreds of clinicians, including ICPs and physicians, reported suspicious illnesses during the surveillance period. In the public sector, one to three epidemiologists from the NJDHSS and CDC team reviewed reports daily and entered them into a database on the day they were received. These epidemiologists then determined which reports were forwarded to the clinical investigation team for additional follow-up.

Two to three other physician epidemiologists followed up with physicians and ICPs to determine which of these patients warranted more definitive testing at the state or CDC laboratories. These physicians provided consultative information on clinical questions related to anthrax and instructed community clinicians on laboratory testing protocols.

Finally, one epidemiologist managed laboratory matters, including arrangements for transporting, tracking, and updating results for specimens. Numerous state and CDC lab staff performed testing; several epidemiologists in Atlanta helped report and interpret CDC testing results.

A total of 37 (61%) ICPs from the 61 hospitals participating in stimulated surveillance completed a survey to describe the resources available to hospitals and to assess experiences with surveillance activities. Most respondents represented community-based hospitals, with <100 beds and at least one full-time ICP. All hospitals responding in the survey had both e-mail and fax capacity; 35 (95%) ICPs received surveillance information by either fax or e-mail; the remainder received information through telephone or other contact.

Before modifications to reporting, 21 (57%) ICPs reported that each daily summary report took 0.5–1 h to complete; 10 (26%) spent 1–3 h; and 5 (13%) spent >3 h. Nine (24%) respondents stated the initial criteria were broad and included many persons with illnesses not attributable to anthrax (e.g., asthma, congestive heart failure). After modifications, 30 (81%) ICPs spent 0.5–1 h completing daily summary reports; 7 (18%) spent 1–3 h.

Medical Examiner Data

During the surveillance period, only one unexplained death after September 1 was reported to the state medical examiner. The patient, a 44-year-old woman with a smoking history and several days of nonfebrile respiratory illness, died on October 14; chest radiographs and blood and sputum cultures were negative. She had been unemployed and had no history of mail handling. No samples were available for additional testing, and no additional follow-up was needed.

Discussion

Intensive and comprehensive surveillance was an essential component of the national response to the crisis precipitated by this event. In New Jersey, the source of all recognized letters containing *B. anthracis*, we implemented surveillance for clinical syndromes compatible with inhalational or cutaneous anthrax over a wide geographic area representing a large population base. The information gathered through this surveillance was pivotal in documenting the relatively limited scope of the outbreak-associated anthrax cases, which in turn confirmed that exposures sufficient to cause disease occurred primarily among persons with occupational exposure to mail processed by one distribution center.

Surveillance efforts were successful in engaging hospitals and health-care providers to identify and report patients with clinical syndromes compatible with inhalational or cutaneous anthrax. We were able to investigate the etiologies of these patients' illnesses and document that additional cases of anthrax did not occur. This finding, in the context of a comprehensive surveillance system, helped to characterize the outbreak, demonstrating that it was confined to the originally recognized cases and confirming that the risk of developing illness in the general population was low. The finding also provided a level of assurance that cases due to this bioterrorist attack, as well as possible additional attacks on other mail processing centers in the area, were not occurring and confirmed that additional public health control measures were not needed.

This surveillance program included several successful elements. The mobilization of state and local health departments in regional efforts allowed for the monitoring of a large geographic area and fostered cooperation among the jurisdictions. We involved hospital-based surveillance participants by providing feedback and soliciting their input, and the system became more efficient with modifications implemented in response. Surveillance heightened awareness among the practicing physicians, and their cooperation allowed for timely reporting and efficient clinical follow-up.

Because all acute-care hospitals in the selected areas participated fully, providing us with reports of many patients with the defined clinical syndromes, cases in the region were likely not missed. In addition, reporting and clinical follow-up were conducted in a timely fashion, which is critical to public health surveillance and response (5,6). Daily summary reports were usually received within 1 to 2 days of the date of the reported data; longer lag times or missed reports occurred mainly during weekends. Possible inhalational anthrax reports were usually received within 1 day of the reported date of hospital visit or admission. Follow-up of each possible inhalational anthrax report took up to several days to complete; lag time in most circumstances was attributable to the period of time needed to receive laboratory results.

Our surveillance efforts had several limitations. While hospital-based surveillance was limited to selected counties, numerous reports throughout New Jersey were received. Cases before surveillance implementation may have been missed, but the state medical examiner's retrospective data would have likely captured these possible earlier cases. Finally, surveillance was costly because of demands on personnel in participating hospitals and at the health department, which was dependent on support from CDC personnel assigned to the state during the outbreak period. This intense level of

surveillance was justifiable given the nature of the anthrax emergency, but it was neither necessary nor feasible to sustain in the long term, once information showed that no additional anthrax exposures had occurred and the upper limit of the incubation period had passed. Keeping the resource-intensiveness in mind, the best ways to integrate surveillance of bioterrorist attacks into existing public health systems need to be evaluated.

Because the agents or methods of future bioterrorist attacks cannot be predicted with certainty, planning surveillance to detect a future bioterrorist attack will require that public health departments consider all possible scenarios and develop a multifaceted approach (7). A future attack might be similar to the recent experience with *B. anthracis*, in which astute clinicians reported a small number of cases, illustrating how community health-care providers are integral to successful surveillance efforts. In this scenario, detecting new cases will depend on accurate diagnostics and timely reporting by medical care providers, highlighting the importance of educating practicing clinicians on what to report, how to report, and ultimately how to interface with the public health system. To this end, public health departments should foster education about bioterrorism and surveillance in the medical community and engage key community medical personnel in these educational and surveillance efforts (8). In addition, public health departments should encourage clinicians to report diagnostic clues and patients with illness patterns that might indicate an unusual infectious disease outbreak associated with intentional release of a biologic agent (9). Finally, public health departments should develop the capability to immediately investigate suspicious reports (10). As our experience in New Jersey demonstrates, establishing and maintaining a comprehensive surveillance system in response to a bioterrorist attack is complex and resource-intensive. Once our surveillance system established that the outbreak was not ongoing, sustaining such an intense surveillance effort was not necessary. The greater challenge for public health departments in the United States will be to design sustainable systems that can assist in detecting future outbreaks.

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At the time of this investigation, Dr. Tan was an Epidemic Intelligence Service officer, Epidemiology Program Office, Division of Applied Public Health Training, assigned to the New Jersey Department of Health and Senior Services. Her interests include both communicable and chronic diseases.

References

1. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
2. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
3. Teutsch SM. Considerations in planning a surveillance system. In: Teutsch SM, Churchill RE, editors. Principles and practice of public health surveillance. New York: Oxford University Press, 1994. p. 18–27.
4. Alter MJ, Mares A, Hadler SC, Maynard JE. The effect of underreporting on the apparent incidence and epidemiology of acute viral hepatitis. Am J Epidemiol 1987;125:133–9.
5. Centers for Disease Control and Prevention. Updated guidelines for evaluating public health surveillance systems: recommendations from the Guidelines Working Group. MMWR Morb Mortal Wkly Rep 2001;50(RR13):1–35.
6. Klaucke DN. Evaluating public health surveillance. In: Teutsch SM, Churchill RE, editors. Principles and practice of public health surveillance. New York: Oxford University Press, 1994. p. 158–72.
7. Centers for Disease Control and Prevention. Biological and chemical terrorism: strategic planning preparedness and response. MMWR Morb Mortal Wkly Rep 2000;49(RR04):1–4.
8. Centers for Disease Control and Prevention. Addressing emerging infectious disease threats: a prevention strategy for the United States. Atlanta: U.S. Department of Health and Human Services, Public Health Service, 1994.
9. Centers for Disease Control and Prevention. Recognition of illness associated with the intentional release of a biologic agent. MMWR Morb Mortal Wkly Rep 2001;50:893–7.
10. 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;32:277–82.

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Bioterrorism-Related Anthrax Surveillance, Connecticut, September–December, 2001

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On November 19, 2001, a case of inhalational anthrax was identified in a 94-year-old Connecticut woman, who later died. We conducted intensive surveillance for additional anthrax cases, which included collecting data from hospitals, emergency departments, private practitioners, death certificates, postal facilities, veterinarians, and the state medical examiner. No additional cases of anthrax were identified. The absence of additional anthrax cases argued against an intentional environmental release of *Bacillus anthracis* in Connecticut and suggested that, if the source of anthrax had been cross-contaminated mail, the risk for anthrax in this setting was very low. This surveillance system provides a model that can be adapted for use in similar emergency settings.

In response to the World Trade Center attack on September 11, 2001, the Connecticut Department of Public Health, assisted by all Connecticut hospitals, implemented a syndromic surveillance system that monitored admissions to acute-care hospitals and visits to emergency departments to detect any concurrent bioterrorism event. All hospitals and emergency departments were asked to report six categories of admissions: respiratory conditions of any type, pneumonia, meningitis or encephalitis, paralysis or paresis of nontraumatic origin, clusters of unusual illness, and total admissions on a daily basis.

After the first confirmed inhalational anthrax case on October 4 (1), the surveillance system was modified to detect the early phase of any disease outbreak that might occur as a result of mass exposure to biological agents—bacteria, viruses, or toxins—used for terrorism. Seven additional hospital admission categories were included in the surveillance system: hemoptysis, acute respiratory distress syndrome or respiratory failure of uncertain origin, sepsis or nontraumatic shock, fever and rash, fever of unknown origin, gastrointestinal symptoms (vomiting, diarrhea, and dehydration), and skin infections.

On November 20, 2001, the 11th known case of bioterrorism-related inhalational anthrax since October 4 was identified in a 94-year-old resident of Oxford, Connecticut, a rural community of <10,000 persons. Unlike most recent patients with bioterrorism-associated anthrax, this patient was not a media or postal worker (1–4). A team of public health investigators from the Centers of Disease Control and Prevention was invited by the state of Connecticut to work in collaboration with state and local health officials to conduct an epidemiologic investigation.

After the death of the index patient on November 21, ongoing statewide surveillance for bioterrorism-related disease was expanded to meet two objectives: 1) conduct retrospective surveillance to identify any previously undetected cases of anthrax since September 1, 2001, that might provide clues to the source of exposure and to assess the possibility of intentional environmental release of *Bacillus anthracis* and 2) conduct prospective surveillance to detect early cases of anthrax that might occur and ensure rapid detection and treatment. The surveillance activities that occurred during this epidemiologic investigation are described.

Methods

Surveillance Activities

Because the first identified case of bioterrorism-related human anthrax in the United States (1) had a presumed source of exposure in mid-September 2001, retrospective surveillance focused on the period from September 1 to November 30, 2001. Methods included reviewing death certificates, laboratory data, medical examiner's records, and postal worker

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absentee records to find evidence of illness in the general population and conducting a veterinary survey to seek evidence in animal populations. Prospective surveillance focused on the period beginning November 21, 2001, and included hospital admissions, emergency department visits, and private physician reports. After retrospective surveillance was completed, we also initiated prospective surveillance of medical examiner and postal worker absentee records.

Case Definitions

We defined a confirmed case of anthrax as clinically compatible illness in a person with laboratory confirmation by isolation of *B. anthracis* from a clinical specimen or other laboratory evidence of *B. anthracis* infection based on at least two supportive laboratory tests (e.g., polymerase chain reaction or serologic or immunohistochemical testing). We defined a suspected case as a clinically compatible case of illness without isolation of *B. anthracis* and no alternative diagnosis, but with laboratory evidence of *B. anthracis* by one supportive laboratory test; or a clinically compatible case of anthrax linked by epidemiologic methods to a confirmed environmental exposure but without corroborative laboratory evidence of infection. Illnesses that were investigated and failed to fulfill criteria for the above case definitions were classified as "no apparent anthrax disease."

Retrospective Surveillance

Death Certificates

All death certificates for persons who died in Connecticut from September 1 to November 30 were reviewed to ascertain if any deaths could be potentially associated with anthrax. Because of the central role of contaminated letters in previous anthrax cases, surveillance focused on deaths occurring in Oxford, where the patient lived, and the eight surrounding towns (Ansonia, Beacon Falls, Derby, Naugatuck, Seymour, Shelton, Southbury, and Woodbury [total population 152,481]) served by the same postal processing and distribution center in Wallingford, Connecticut. This facility received mail from postal distribution facilities known to be contaminated by *B. anthracis* spores, including the postal center in Hamilton, New Jersey, where the envelopes containing *B. anthracis* sent to two U.S. senators originated.

Death certificates with the following conditions listed as the immediate or underlying cause of death were selected for further review: pneumonia, sepsis, cardiac arrest without cause, respiratory arrest without cause, sudden death, and undetermined cause. Deaths were further classified by place of occurrence: hospital, nursing home, residence, or other setting. Because of the paucity of clinical information on deaths occurring outside hospitals, the review focused on in-hospital deaths.

To obtain additional information on in-hospital deaths, laboratories, infection control practitioners, and physicians were contacted by telephone to identify patients for whom a definitive cause of death could be determined. For the remaining deaths in which cause of death could not be ascertained, medical record reviews by a team of four physician epidemiologists using a standardized abstraction form were conducted at the hospitals where the deaths occurred.

Laboratory Data

Hospital-associated laboratories statewide were contacted to obtain information on any gram-positive rods or *Bacillus* species isolated from sterile sites (e.g., blood, cerebrospinal fluid, or pleural fluid). A standardized reporting form was provided to laboratories to be completed and sent to a 24-hour–accessible fax machine. For *Bacillus* species isolates, we contacted laboratories by phone to gather information about motility and hemolysis tests when this information was not provided on the report. For all other reports of gram-positive bacilli, laboratories were contacted to obtain speciation information if available, when this information was not provided. All available isolates suspicious for *B. anthracis* were sent to the Connecticut Department of Public Health (CDPH) laboratory for final identification.

Medical Examiner's Records

Connecticut's state medical examiner is notified of deaths that occur outside hospitals or within 24 hours of hospitalization. Data on deaths referred to the medical examiner and reported from September 1 to November 26 were reviewed. After November 26, ongoing prospective surveillance for deaths referred to the medical examiner was assumed by CDPH, with a particular focus on deaths in the town where the index patient resided and the eight surrounding towns. The medical examiner's office and CDPH made the decision about whether an autopsy was necessary to exclude anthrax as the cause of death, based on the symptoms of the deceased patient and the clinical circumstances surrounding death.

Postal Worker Absenteeism

Work attendance records were obtained from both the local postal and main processing distribution facilities serving the index patient's town of residence and the eight surrounding towns (Seymour and Wallingford postal facilities). To obtain information about reasons for absence, either postal management or CDPH personnel interviewed postal workers with absences for $\geq$3 consecutive days from September 11 to November 25, 2001. When workers were not available to be interviewed, information was obtained by interviewing management personnel, who also were questioned about recent deaths in postal workers.

Surveillance for Postal Worker Influenzalike Illness and Cutaneous Conditions

The U.S. Postal Service had been conducting surveillance for influenzalike illness or cutaneous conditions compatible with anthrax among postal workers nationwide since October 25, 2001. In Connecticut, postal service management collected

data from postal workers and reported to the postal medical office in Hartford, Connecticut. Reports from employees were voluntary. Data for the state were submitted to area headquarters (serving New England and parts of New York) daily and then reported to national postal headquarters in Washington, D.C. For cases in which the postal worker was hospitalized with influenzalike symptoms, national headquarters was notified directly. Beginning November 6, only hospitalizations were reported to area headquarters; however, data for the state of Connecticut were still collected in Hartford. All past reports to the system and ongoing reports were reviewed to characterize the symptoms and signs; for conditions suspicious for anthrax, health-care providers were called for further clinical information.

Prospective Surveillance

Hospital, Emergency Departments, and Physician Reports

The statewide hospital-based surveillance for bioterrorism-related agents that began after September 11, 2001, was enhanced from November 27 to December 15. All acute-care hospitals in Connecticut designated a surveillance officer (e.g., infection control practitioner, nurse, or physician) who would be responsible for surveillance of conditions potentially related to anthrax and other bioterrorism-related agents at their institution. Each day, the surveillance officer contacted the clinical microbiology laboratory to request a list of any suspect Gram stain results or bacterial isolates from sterile sites. Suspect results were defined as gram-positive rods that had not been further identified or *Bacillus* species that had not been further typed or for which speciation as *B. anthracis* had not been excluded. Additionally, the surveillance officer reviewed admissions for the previous 24 h and reported patients having any one of five clinical syndromes (acute respiratory failure with pleural effusion; hemorrhagic enteritis with fever; a skin lesion characterized by vesicles, ulcer, or eschar; meningitis, encephalitis, or unexplained acute encephalopathy; or anthrax or suspected anthrax infection) and a widened mediastinum on chest radiograph or laboratory findings of a gram-positive bacillus on Gram stain, *Bacillus* species from culture of a sterile site specimen, or hemorrhagic cerebrospinal fluid, pleural, or peritoneal fluid in patients without a traumatic tap or event.

Using a standardized form, the surveillance officer reported findings daily to CDPH. Upon identifying patients with the surveillance criteria for a suspect anthrax case, hospital surveillance officers contacted a designated member of the surveillance team by telephone and faxed the report. These patients were then referred to a clinical team for further evaluation. In addition, physicians and infection control practitioners statewide (in particular those in the nine towns including and surrounding the town of the index patient) were asked to report immediately to CDPH any patient with symptoms that suggested anthrax.

Other Anthrax Surveillance Activities

Survey of Veterinary Practices

To ascertain undiagnosed animal anthrax cases, a one-page questionnaire was distributed to the members of the Connecticut Veterinary Medical Association (CVMA) on November 28. CVMA has a total of 620 members, accounting for 82% of the 768 CDPH-licensed veterinarians in Connecticut. Information collected included the number of veterinarians associated with the practice, type of practice, number of undiagnosed deaths by animal species, animal deaths accompanied by clinical signs consistent with anthrax, and knowledge of confirmed cases of animal anthrax in Connecticut. Questionnaires were sent by the CVMA rapid fax system to the approximately 325 members who requested faxed updates from CDPH. We requested a single completed questionnaire from each practice. Since some practices included veterinarians who are not CVMA members, the survey likely reached more veterinarians than actual members who had requested faxed updates.

Results

Data were entered and analyzed in an Epi Info database (5). Hospital, emergency department, and physician reports were evaluated at least twice a day.

Among the 487 deaths reported from the nine towns in September, October, and November 2001, a total of 131 (26.9 %) had one of the six conditions under surveillance. Of these, 66 (50.3%) occurred in hospitals; the rest occurred in residences, nursing homes, and other settings. No postmortem examinations were performed. By contacting physicians, infection control practitioners, and laboratories, a likely cause of death other than anthrax was identified for 7 (10.6%) patients. For the remaining 59 (89.4%) patients, medical record review was necessary. In 33 (55.9%), a cause of death other than anthrax was identified. For 12 (20.3%) patients, the cause of death was not apparent, but available information on the clinical features and clinical course (such as absence of fever and respiratory symptoms) of the patients did not suggest a diagnosis of anthrax. Insufficient data were available to assess the cause of death for 14 (23.7%) patients because death occurred before or shortly after arrival to the hospital. None of these patients had been autopsied, and because of the lack of a clear indication and the limited availability of resources, no further measures (e.g., exhuming the body to conduct autopsy) were taken to ascertain the cause of death.

Laboratory Data

Thirty (96.7%) of 31 clinical laboratories provided data. Twenty-two (73.3%) laboratories reported at least one patient with a gram-positive bacillus or *Bacillus* species isolate. Gram-positive bacilli were identified in 71 specimens from 70 patients (one patient had more than one specimen submitted), including blood (59 specimens), tissue (6 specimens), perito-

neal fluid (3 specimens), pleural fluid (2 specimens), and 1 surgical site specimen. Of patients with gram-positive bacilli, 49 had *Bacillus* species isolated; none of these was identified as *B. anthracis*. For the remaining 22 reports of gram-positive bacilli, 16 were identified as *Corynebacterium*, 1 as *Propionobacterium*, 1 as *Clostridium*, 1 as *Eubacterium*, and 1 as *Staphylococcus hominis*; 1 was a mixed infection with gram-positive organisms, and 1 was an unidentified motile gram-variable bacillus.

Medical Examiner's Records

One hundred forty-eight deaths were reported to the medical examiner. Of these, autopsies were performed on 14 (9.4%) patients. Cause of death was determined to be an accident in six, cardiac disease in four, suicide in three, and inhalational anthrax in one (the index patient). Because of the lack of clinical information on the remaining patients who had not been autopsied, further review was not possible.

Postal Worker Absenteeism

At the local postal facility in Seymour, no employees died during the surveillance period. Two persons were absent for ≥3 days, one for a scheduled surgery and the other for an injury. At the main processing and distribution center in Wallingford, two recent postal worker deaths were attributed to cardiovascular disease; both occurred before September 11, 2001. Approximately 35 employees were absent for >3 consecutive days. Interviews of the postal workers about the reasons for absence showed no apparent anthrax in any workers.

Postal Worker Influenzalike Illness and Cutaneous Lesion Surveillance

Ninety-two reports of influenzalike illness were reviewed. For seven patients with characteristics that might have been compatible with anthrax (e.g., cutaneous lesions, influenzalike illness with absence of rhinorrhea, and shortness of breath), further clinical information was obtained. All cases were classified as "no apparent anthrax disease" after review.

Prospective Surveillance

Hospital, Emergency Departments, and Physician Reports

Of 59 reports received, all were classified as "no apparent anthrax disease." Specimens from 14 patients were sent to CDC, including 15 serum specimens, 14 skin biopsy specimens, 3 lung biopsies, 2 samples of pleural fluid, 11 samples of whole blood for polymerase chain reaction, and (from one patient) autopsy specimens from the gastrointestinal tract, liver, a lymph node, and one mixed tissue specimen.

Other Anthrax Surveillance Activities

Survey of Veterinary Practices

A total of 140 questionnaires were returned from 140 practices, representing 365 veterinarians and 48% of licensed Connecticut veterinarians. Completed questionnaires were received from practices distributed throughout eight counties of the state. Of these, 113 (81%) were small animal practices; 14 (10%) a mixture of small animals, equine, and food animal practices; and 12 (9%) equine practices. Of the respondents, 69 practices with 180 veterinarians, including nine practices and 20 livestock veterinarians, were located in the two counties representing the nine towns of interest during surveillance. Of the 140 practices, 18 (13%) reported that they were aware of undiagnosed animal deaths since September 15, 2001. None of the respondents indicated that they or anyone in their practice knew of a confirmed case of animal anthrax in Connecticut.

Discussion

Despite intensive, active prospective and retrospective surveillance, we did not identify any patients other than the index case with features compatible with anthrax. This finding indicates that the index patient was probably not exposed through intentional local environmental release of *B. anthracis;* therefore, the concurrent epidemiologic investigation focused on the personal activities and contacts of the patient. Our findings, in conjunction with the *B. anthracis* contamination of the regional postal distribution facility, suggest that the index patient was likely exposed through cross-contaminated mail. If so, the lack of additional anthrax cases among persons who received mail from the same postal facility as the index patient also suggests that the risk from cross-contaminated mail in this setting was very low.

The scope of this epidemiologic investigation did not include a formal evaluation of the surveillance system. Although a standard for evaluating the performance of a system to detect covert acts of bioterrorism has not yet been described, we have some general comments about the traditional criteria (6) used in assessing the attributes of surveillance systems. Our system was complex and labor-intensive, requiring an estimated 1,500 person-hours for state and federal public health officials alone during the most intense 3-week period of the investigation. However, the system operated effectively. The acceptance of the system and compliance in reporting were likely enhanced by both national and local events—the World Trade Center and Pentagon attacks, the subsequent anthrax–tainted mailings, and the death of the Connecticut resident from anthrax. The staff at the public health department were highly motivated, and training requirements were minimal because of their knowledge of the preexisting system for syndromic surveillance. Use of existing resources provided a foundation for successfully implementing enhanced surveillance in less than 12 hours. Because of standardized and relatively simple reporting forms and data abstraction by trained investigators, quality of the data was excellent. The system was by design flexible and met evolving needs, including adding new syndromes to the surveillance system and moving staff from one activity to another as needed. Centralized reporting by fax or telephone assisted us

in identifying early any problems in implementation of the surveillance system.

The true frequency of reportable syndromes was not known before we implemented this surveillance system for bioterrorism-related agents. Furthermore, with no prior knowledge of bioterrorism events, adequate numerator for the occurrence of any bioterrorism-related syndrome, or denominator for the population susceptible to the event, calculating the sensitivity and predictive value positive for the system was difficult. However, this system likely reflected accurately the lack of additional anthrax cases in both animal and human populations in Connecticut. Approximately 80% of Connecticut-licensed veterinarians in the state were successfully surveyed, including veterinarians who treat livestock most susceptible to anthrax infection, and none reported any animal illness consistent with anthrax. Similarly, an exhaustive search for human anthrax cases based on review of clinical and laboratory data yielded no additional cases.

In general, we received timely data that ensured quick and appropriate public health responses and allowed modifications to the system as needed. For hospital reporting, most reports were transmitted to a designated fax by noon each day for events during the preceding day. This plan was not problematic except on weekends, when hospitals were often operating with minimal staff. Without exception, all hospitals submitted data no later than 4 p.m. on the day of required reporting. Frequently, hospital, laboratory, medical examiner, and postal service personnel contacted a member of the team by telephone or pager with concerns about potential patients with suspect symptoms. The Connecticut Vital Records Department directed the daily transmission of all death certificates from the towns of interest, which allowed for continual monitoring of suspected deaths that required further investigation. The surveillance system operated 24 hours a day, 7 days a week; surveillance team members were always available. This constant accessibility was helpful with data turnaround and evaluation of suspect cases but difficult to sustain and resource intensive. Although surveillance instruments evolved over time, these changes did not detract from the ability to collect, manage, and disseminate the data, attesting to the stability of the system.

Our surveillance activities met the objectives of providing information about the source of exposure for the index case and guiding the course of the accompanying epidemiologic investigation. Although we were able to approach "real-time" reporting, permanent sustainability of these activities is unrealistic because they require too many resources. While the costs of sustaining this system were not directly evaluated, such an analysis would be useful. Explicit discussion of costs and benefits may help both in terms of protecting and increasing funding levels and assuring that existing surveillance systems are necessary and make the best possible use of limited resources. In situations requiring surveillance, an approach similar to ours could be applied after suitable modifications to meet the need for short periods of time. Clearly, the approaches to detecting sentinel bioterrorism events require further evaluation, standardization, and improvements to allow a timely, efficient, and effective public health response.

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Dr. Williams is an Epidemic Intelligence Service officer assigned to the New Hampshire Department of Health and Human Services. She was a member of the Connecticut Anthrax Investigation Team.

References

1. Centers for Disease Control and Prevention. Notice to readers: ongoing investigation of anthrax—Florida, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:877.
2. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
3. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
4. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001:50:889–93.
5. Dean AG, Dean JA, Coulombier D, Burton AH, Brendel KA, Smith DC, et al. Epi Info, version 6: a word processing, database, and statistics program for public health on IBM-compatible microcomputers. Atlanta: Centers for Disease Control and Prevention; 1995.
6. Centers for Disease Control and Prevention. Updated guidelines for evaluating the public health surveillance systems. MMWR Morb Mortal Wkly Rep 2001;50:1–35.

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Environmental Sampling for Spores of *Bacillus anthracis*

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On November 11, 2001, following the bioterrorism-related anthrax attacks, the U.S. Postal Service collected samples at the Southern Connecticut Processing and Distribution Center; all samples were negative for *Bacillus anthracis*. After a patient in Connecticut died from inhalational anthrax on November 19, the center was sampled again on November 21 and 25 by using dry and wet swabs. All samples were again negative for *B. anthracis*. On November 28, guided by information from epidemiologic investigation, we sampled the site extensively with wet wipes and surface vacuum sock samples (using HEPA vacuum). Of 212 samples, 6 (3%) were positive, including one from a highly contaminated sorter. Subsequently *B. anthracis* was also detected in mail-sorting bins used for the patient's carrier route. These results suggest cross-contaminated mail as a possible source of anthrax for the inhalational anthrax patient in Connecticut. In future such investigations, extensive sampling guided by epidemiologic data is imperative.

Following the bioterrorism-related anthrax attacks in October 2001, a total of 22 cases of anthrax were identified: 11 confirmed cases of inhalational anthrax, and 11 (7 confirmed and 4 suspected) cases of cutaneous anthrax (1). Epidemiologic investigation of the first nine patients with inhalational anthrax showed that they were exposed to particulate aerosols containing *Bacillus anthracis* when they opened letters or when letters were processed in postal facilities (2).

The final case of inhalational anthrax in 2001, reported on November 19, was in a 94-year-old woman from Oxford, Connecticut, who died (3). Unlike previous cases, the patient was not a postal employee, mail handler, media worker, or government official (1,2). An extensive investigation for *B. anthracis* spores was conducted at her home and other places that she visited in the 2 months preceding her death; all samples were negative (4). Retrospective and prospective surveillance detected no additional cases of anthrax in her community (5,6), and an intentional release of anthrax spores there was considered unlikely. The investigation focused on mail as the source of anthrax; we subsequently conducted intensive sampling of the postal facility that serves her region. We describe the sampling methods, results, and public health implications of repeated environmental sampling in this facility.

The Setting

The regional postal processing center for the patient is the Southern Connecticut Processing and Distribution Center (SCPDC) in Wallingford. With a floor area of 350,000 square feet and the capacity to process up to 3 million pieces of mail a day, the center is in operation around the clock. In November 2001, SCPDC employed 1,122 workers.

The center is equipped with 6 advanced-facer canceller machines, 5 optical character reader machines, 5 bar-code sorting machines, and 13 digital bar-code sorting (DBCS) machines for processing letters. In addition, automated flat sorting machines, linear integrated parcel sorters, and small bundle and parcel sorters are used to process flats (large flat pieces of mail that are not packages) and parcels (wrapped packages). Although all these machines are part of the facility, they differ in function, speed of processing, and location within the facility.

Mail Processing

The advanced facer-canceller machines cancel letters originating from southern Connecticut and apply two bar codes that are used to identify and sort letters for their final destination. The identification tag, a fluorescent orange bar code on the back of the envelope, records the time and date that the letter was canceled. The postnet barcode, a series of vertical full and half bars applied to the front of an envelope, contains zip code and delivery point information in machine-readable format. Advanced facer-canceller machines are used primarily to process stamped mail; bulk letters are not processed on canceling machines because they already have barcodes applied by the mailers and are presorted.

The high-speed computerized DBCS machines are used for preliminary and final sorting of the mail by barcode. During the preliminary sort, letters can be processed on any one of

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the 13 DBCS machines at the facility. This step arranges the letters by the 5-digit zip code of the delivery address, usually requiring ≤2 passes to sort a batch of mail. Once this step is accomplished, mail is transported for final processing to a designated DBCS machine, which sorts the letters to the 9- or 11-digit zip code, usually requiring ≤3 passes. Therefore, letters addressed to the patient could have been processed initially on any of the 13 DBCS machines. Later, the final sort would have been processed on DBCS no. 6, where specific bins were designated for the carrier route.

In October and November 2001, independent contractors working for the U.S. Postal Service (USPS) tested postal processing and distribution plants nationwide to determine if any had become contaminated with *B. anthracis* following the bioterrorism events. As part of this effort, SCPDC was tested on November 11, 2001; all results were negative for *B. anthracis* contamination. Following the report of the inhalational anthrax case in Oxford, Connecticut, the facility was tested again extensively.

Methods

Samples were obtained from SCPDC on November 11, 21, 25, and 28 and December 2. Sampling methods included dry swabs, wet swabs, wet wipes, and HEPA vacuum.

On November 11, a contracting company working for USPS obtained samples from SCPDC as part of the nationwide testing of postal facilities for anthrax spores. The contractor took dry synthetic swabs from random sites in the facility and sent them to be analyzed at the Connecticut Department of Public Health Laboratory. On November 21, 2001, after the report of the 94-year-old woman with anthrax in Connecticut, a second independent contractor hired by USPS collected additional dry swab samples from surfaces where letters, flats, and parcels were processed. These samples, along with others collected from air circulating units, were analyzed by the Connecticut Department of Public Health Laboratory.

On November 25, the investigation team obtained samples from the facility using wet synthetic swabs and processed them by methods recommended by CDC (7,8). Samples were taken from the letter canceling and sorting machines, flat and parcel sorting machines, and five facility vacuum filters in use since October 27, 2001. The samples were analyzed by the Connecticut Department of Public Health Laboratory.

Samples taken on November 28 were more extensive. Guided by additional epidemiologic data, we collected samples from carefully selected sites (the canceling and sorting machines) by using wet synthetic 2x2-inch wipes and HEPA vacuum. Specimens were collected and transported according to recommended methods (7,8). Wipe and vacuum samples were cultured and analyzed at a CDC-contracted laboratory.

On December 2, following the first report of anthrax-positive results in the facility, we collected follow-up samples. A composite sample from the vertical column of four bins was taken from all columns on the four DBCS machines that were presumptively positive based on sampling done on November 28. These wet wipe samples, taken to determine the extent of contamination on the machines, were analyzed by a CDC-contracted laboratory.

Results

A total of 589 samples were collected from November 11 to December 2, 2001. Three hundred forty-six (59%) of these were from the DBCS machines. Of the 589 samples, 117 (20%) were dry swabs, 60 (10%) wet swabs, 300 (51%) wet wipes, and 112 (19%) HEPA vacuum samples.

Fifty-three dry synthetic swab samples were taken on November 11. Of these, only one (2%) sample was from a DBCS machine (no. 6). All samples were negative for *B. anthracis* (Tables 1,2).

On November 21, 64 dry synthetic swab samples were taken. Of these, six (10%) were from the DBCS machines, two each from DBCS nos. 5, 6, and 7. All samples were negative for *B. anthracis* (Tables 1,2).

On November 25, the investigation team took a total of 60 wet synthetic swab samples; 8 (13%) were from the DBCS machines. Of the eight samples taken from the DBCS machines, one sample each was taken from DBCS nos. 1, 2, 9, 11, and 13 and three from DBCS no. 6. All samples were negative for *B. anthracis* (Tables 1,2).

On November 28, the most extensive sampling was conducted, with 212 samples collected. Of these, 102 (48%) were wet wipes and 110 (52%) vacuum samples. We used wet wipes for sampling the stacker bins (hard surfaces) and the HEPA vacuum for sampling the machines, including the inaccessible parts. We focused our sample collection on machines likely to have processed mail delivered to the patient's address. Although all machines were tested, 131 (62%) samples were from DBCS machines, which processed both stamped mail and nearly all the bulk presorted mail; approximately 80% of the mail recovered from the patient's home was bulk mail.

Of 212 samples, 6 (3%) yielded *B. anthracis*, and all positive samples were from DBCS machines. Of the six anthrax-positive samples, two were vacuum samples from DBCS nos. 4 and 10, and four were wet wipe samples from the bins of DBCS machines nos. 10 and 11. One vacuum sample (0.55 g of specimen) from the feeder part of machine no. 10 had 2.9×10^6 CFU of *B. anthracis*, equal to 5.5×10^6 CFU of *B. anthracis* per gram of sample material. Of the mail sorted on this machine, approximately 75% is bulk mail. This machine had not been sampled before November 28, the fourth round of sampling.

Following the results of the sampling on November 28, we collected follow-up samples on December 2. We took samples to determine the extent of contamination on DBCS machines nos. 4, 10, and 11, the machines from which results were positive for *B. anthracis* on the November 28 sampling. In addition, we also collected samples from DBCS machine no. 6 because preliminary positive results from the November 28 sampling were reported and because this machine was used for

Table 1. Number of samples taken from digital bar-code sorting machines during five sampling dates, Connecticut, 2001

Machine no.	11/11/01	11/21/01	11/25/01	11/28/01	12/02/01	Total samples
1			1	8		9
2			1	8		9
3				8		8
4				11[a]	48[a]	59
5		2		12		14
6	1	2	3	23	48[a]	77
7		2		12		14
8				8		8
9			1	8		9
10				8[b]	52[c]	60
11			1	8[a]	52[d]	61
12				8		8
13			1	8		9
Total	1	6	8	130	200	345

[a]One positive sample.
[b]Four positive samples.
[c]Thirty positive samples.
[d]Three positive samples.

final processing of mail to the address of the patient. The 200 wet wipe samples taken on December 2 were composite wipes from a vertical column of four bins from each machine (each machine has 48–52 columns of four bins). We collected composite samples to allow complete sampling of all bins from all suspect machines without taking an excessive number of samples (Table 2).

Of 200 composite column samples from DBCS machines nos. 4, 6, 10, and 11, a total of 35 (17.5%) columns of bins were positive. On machine no. 10, 30 (68%) of 52 columns were positive. Three (6%) of 52 columns from machine no. 11 and 1 (2%) of 48 columns on both machines no. 4 and 6 were positive. These results confirmed the high contamination of machine no. 10. Only 1 of 48 columns of bins on machine no. 6 was found to be positive. Machine no. 6 was used for final mail sorting for several zip codes including the town where the patient lived. The only column of bins that yielded *B. anthracis* on DBCS no. 6 included bins for the carrier route for the patient's home.

Discussion

Supplemented by the findings of the epidemiologic investigation team, our investigation identified cross-contaminated mail as a possible source of anthrax for the Connecticut patient (4). No other source of contamination in her community was identified after extensive sampling of her home and areas she visited; no other cases of anthrax were reported. We identified a contaminated sorting machine that was used to sort most of the mail delivered to the patient, including bulk mail; the specific column of bins that held mail for her carrier route was positive (4). Extensive sampling with large numbers of samples was required to find anthrax spores. Positive results were obtained following sample collection based on information learned during the epidemiologic investigation. All positive results were obtained from samples collected by using wet wipes and vacuum sampling. All the dry or wet swab samples were negative for *B. anthracis*.

Environmental sampling during an anthrax investigation is critical in determining the likely source of infection and the extent and degree of environmental contamination, to support decisions on the need for prophylaxis with antibiotics or clean-up, and to provide guidance about when clean-up is adequate to permit reentry into an area. During this investigation, no validated methods for specifically sampling the environment for *B. anthracis* were known. We lacked data on the effectiveness of the sample collection media (swabs, wipes, and vacuum) for typical porous and nonporous surfaces encountered in indoor environments. The effect of varying concentrations of *B. anthracis*–containing particles and dust loading on sampling efficiency had also not been studied. Furthermore, recovery efficiency of the analytical methods (efficiency of removal of *B. anthracis* spores from the sample collection media) had not been adequately evaluated, and limits of detection have not been established (8).

Although our investigation showed that different sample collection techniques and sampling sites and numbers of samples yielded different findings, results are based on observation and cannot be used to specifically compare the different approaches. However, exploring the reasons for the different results may be useful for future investigations. On November 11, all samples were collected by using dry swabs from random sites in the facility with the intent of finding contamination anywhere in the facility. Only one sample from the DBCS machines was taken. On November 21, more samples were taken from the DBCS machines, but still only three machines were sampled. This sampling was performed with emphasis on the Oxford mail route because the illness had been reported in that community. However, whether the patient's mail was predominantly bulk mail and whether letters could have been sorted preliminarily on any DBCS machine were not known at the time. The November 25 sampling was similar to the November 21 sampling except that investigators used wet swabs instead of dry swabs. Again, limited samples from six DBCS machines were taken.

On November 28, more extensive and directed sampling was conducted, and epidemiologic information was available to guide us to the appropriate sites. Using wet wipes and HEPA vacuum led to the first positive results for anthrax in the facility. A recent study, conducted after the Connecticut investigation, has confirmed our findings (WT Sanderson et al., unpub. data). In this study, side-by-side surface swabs, wipes, and HEPA vacuum samples were taken at the Brentwood Processing and Distribution Center in Washington, D.C., to compare their relative effectiveness in a contaminated postal facility. Wet wipes and vacuum sampling were found to be

Table 2. Environmental sampling methods, types, and results of samples taken November 11–December 2, Southern Connecticut Processing and Distribution Center, 2001[a]

Sampling date	No. of samples	Samples from DBCS	Type	Positive results	Sample collectors
11/11/01	53	1	Dry swabs	0	USPS
11/21/01	64	6	Dry swabs	0	USPS
11/25/01	60	8	Wet swabs	0	CDC/ATSDR
11/28/01	212	131	Wet wipes and vacuum	6	CDC/ATSDR
12/02/01	200	200	Wet wipes	35	CDC/ATSDR
Total	589	346		41	

[a]DBCS, digital bar-code sorting; USPS, United States Postal Service; CDC, Centers for Disease Control and Prevention; ATSDR, Agency for Toxic Substances and Disease Registry.

more effective methods than surface swabs; results from wet wipes and vacuum samples were highly concordant. Of 28 sample locations tested, 4 (13%) were positive with dry swabs, compared with 13 (46%) wet swabs, 23 (82%) wet wipes, and 23 (82%) vacuum samples (WT Sanderson et al., unpub. data).

Although the effectiveness of sampling techniques influences which are used, other factors that determine the choice of sampling techniques include the site of sampling, size of the surface to be sampled, character of the surface (porous or nonporous), need to quantify the results, and preference and specialization of the laboratory where the test is done. Swab samples may still be the best method to sample small hard surfaces not easily accessible for wiping or vacuum sampling (e.g., a keyboard). Surface wipes also have several limitations (8). Wipe samples might miss minimally contaminated surfaces or small, discrete contaminated areas. In addition, sampling all surfaces within a building by using surface wipes is not feasible. Therefore, vacuum samples provide an important tool for maximizing the surfaces that can be evaluated during an investigation (8).

Sampling methods and number of samples are also influenced by the circumstances of the potential contamination. A sufficient number of samples must be taken to increase the probability that the sampling is representative, given the likely extent of contamination. In an initial investigation where a known or suspected release of potentially contaminated material has occurred, the first priority should be to collect samples near the suspected release source (often called directed or targeted sampling). In determining the extent of contamination, investigators should include coverage of areas along an anticipated contaminant pathway, i.e., those associated with air movement or dust collection, as well as activities that result in re-aerosolization or cross-contamination.

When sampling to identify contamination in a facility, the length of time between the suspected contamination of the facility and the time that sampling occurs is also important in determining where and how to collect samples. For example, since the sampling on November 11 was conducted >3 weeks after contamination was probably introduced into the facility, any aerosolized spores of *B. anthracis* had likely already settled on surfaces, and therefore surface sampling, as opposed to air sampling, was reasonable.

The environmental investigation did not identify anthrax spores in the patient's home, possibly because her house was routinely cleaned thoroughly or because the piece of mail that was the source for her infection was not identified. One resident of her community is known to have received an envelope from which *B. anthracis* spores were isolated that was likely to have become cross-contaminated as it passed through the postal system, although no one in that household became ill (2). The patient also probably became ill following exposure to a low number of *B. anthracis* spores, which may explain why she had a relatively long incubation period compared with the other cases reported (9,10). Other host factors, including advanced age, underlying lung disease, medication use (2), and the practice of tearing up bulk mail (4), may have increased her chances of acquiring the disease.

The results of our investigation influenced the adherence and compliance of postal workers on postexposure prophylaxis at SCPDC. A study conducted there showed that 13% of the postal workers stopped taking postexposure prophylaxis because of the initial report of negative environmental cultures in the facility. An increase in postexposure prophylaxis adherence occurred, however, following the positive results in the facility (11).

The reasons why no postal workers at SCPDC became ill during this event are unknown. Perhaps host factors were important or anthrax spores were not aerosolized in sufficient concentration. The finding that spores were not widespread in the facility suggests that the dispersion was likely not due to substantial aerosolization. Following the experience from the Brentwood facility in October 2001, cleaning practices in postal facilities nationwide changed from use of compressed air, which easily aerosolized small particulate materials such as anthrax spores, to use of HEPA vacuums for cleaning (12). At SCPDC, maintenance workers stopped using forced air to clean equipment on October 27, 2001, which may have reduced the time when spores could have been aerosolized. The highly contaminated DBCS machine could have been a source of exposure to postal workers if the cleaning measures had not been changed.

The environmental investigation was central in demonstrating a possible source of infection for the case of inhalational anthrax in Connecticut. Our investigation showed that

extensive sampling was required and that epidemiologic investigation was essential in identifying sites for sampling. None of the dry or wet swab samples were positive. For future investigations of large facilities, we recommend the use of wet wipes and vacuum. Further research is needed to clarify the sensitivity of the sampling and analytical methods for known or suspected *B. anthracis* and to develop clear algorithms for sampling if future investigations are needed. This investigation also demonstrated that illness associated with cross-contaminated mail is a rare but possible phenomenon.

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References

1. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related inhalational anthrax—Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1049.
2. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related inhalational anthrax—Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001:50:1077–9.
3. Barakat LA, Quentzel HL, Jernigan JA, Kirschke DL, Griffith K, Spear SM, et al. Fatal inhalational anthrax in a 94-year-old Connecticut woman. JAMA 2002;287:863–8.
4. Griffith KS, Mead P, Armstrong G, Painter J, Kelley K, Mayo D, et al. Aftermath of Daschle-Leahy: epidemiologic investigation of a case of inhalational anthrax—Connecticut, 2001. In: Program of the 51st Annual Epidemic Intelligence Service Conference, p. 62. Atlanta, Georgia, April 22–26, 2002.
5. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
6. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
7. Centers for Disease Control and Prevention. Evaluation of *B. anthracis* contamination inside the Brentwood mail processing and distribution center—District of Columbia, October 2001; MMWR Morb Mortal Wkly Rep 2001;50:1129–33.
8. Centers for Disease Control and Prevention. Procedures for collecting surface environmental samples for culturing *B. anthracis*. Available from: URL: http://www.bt.cdc.gov/Agent/Anthrax/environmental-sampling-apr2002.asp. Accessed May 24, 2002.
9. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
10. Meselson M, Guillemin J, Hugh-Jones M, Langmuir A, Popova I, Shelokov A, et al. The Sverdlovsk anthrax outbreak of 1979. Science 1994;266:1202–8.
11. Williams JL, Noviello S, Wurtzel H, Griffith K, Hamborsky J, Perz J, et al. Anthrax post-exposure prophylaxis adherence in postal workers. Connecticut, 2001. In: Program of the 51st Annual Epidemic Intelligence Service Conference, p. 74. Atlanta, Georgia, April 22–26, 2002.
12. Centers for Disease Control and Prevention. Notice to readers: interim recommendations for protecting workers from exposure to *B. anthracis* in work sites in which mail is handled or processed. MMWR Morb Mortal Wkly Rep 2001:50;961.

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Call-Tracking Data and the Public Health Response to Bioterrorism-Related Anthrax

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After public notification of confirmed cases of bioterrorism-related anthrax, the Centers for Disease Control and Prevention's Emergency Operations Center responded to 11,063 bioterrorism-related telephone calls from October 8 to November 11, 2001. Most calls were inquiries from the public about anthrax vaccines (58.4%), requests for general information on bioterrorism prevention (14.8%), and use of personal protective equipment (12.0%); 882 telephone calls (8.0%) were referred to the state liaison team for follow-up investigation. Of these, 226 (25.6%) included reports of either illness clinically confirmed to be compatible with anthrax or direct exposure to an environment known to be contaminated with *Bacillus anthracis*. The remaining 656 (74.4%) included no confirmed illness but reported exposures to "suspicious" packages or substances or the receipt of mail through a contaminated facility. Emergency response staff must handle high call volumes following suspected or actual bioterrorist attacks. Standardized health communication protocols that address contact with unknown substances, handling of suspicious mail, and clinical evaluation of suspected cases would allow more efficient follow-up investigations of clinically compatible cases in high-risk groups.

In response to the terrorist attacks on the World Trade Center and the Pentagon in the United States on September 11, 2001, preestablished emergency operations centers at the Centers for Disease Control and Prevention (CDC) were activated to assist in coordinating the public health response. After the first indication of a case of bioterrorism-related anthrax in Florida in October (1–4), the volume of calls to the emergency operations centers from the general public and health departments increased dramatically. In response to this increased demand, the preestablished centers were combined into an agencywide Emergency Operations Center (EOC), specialized teams were established to focus on specific local investigations, and staff was supplemented with additional personnel and resources.

A triage system was established to monitor incoming calls for referral to specialized teams (Figure 1). The State Liaison Team (SLT), which was established as a component of the second tier of this system, was formed to respond to calls from persons reporting illnesses and exposures possibly related to bioterrorism. The SLT assisted with the diagnostic evaluation of illness suspected of being due to anthrax exposure by collecting clinical data, providing information, interpreting recommendations, arranging for diagnostic testing or expert consultation, and facilitating case reporting with state and local health authorities. If highly suspicious illnesses warranted further epidemiologic investigations, the SLT assisted with referrals to field investigation or specialized teams. These teams then coordinated investigation activities with the appropriate state health departments (Figure 1).

We describe the nature and volume of telephone calls received by the EOC, as well as those referred specifically to the SLT for more detailed tracking and follow-up. We use the call data to highlight some implications for staffing strategies and to recommend changes in the EOC triage protocol that may allow second-tier referral teams to focus more exclusively on high-risk case investigations.

Methods

A variety of professional staff screened calls coming into a central telephone bank. A prerecorded message instructed callers to contact their state or local health department if they had not done so. Calls that could be answered with "Frequently Asked Questions" documents or guidelines published in the Morbidity and Mortality Weekly Report (MMWR) were han-

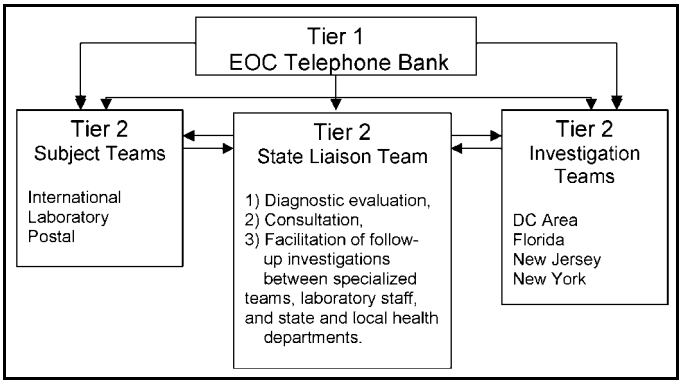

Figure 1. Emergency Operations Center (EOC) telephone call triage system, Washington, D.C. area, October 2001–February 2002

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dled directly by public health advisors, epidemiologists, and junior staff (5–8). Callers were referred to the SLT for follow-up if they reported symptoms consistent with anthrax or other bioterrorism agents, noted exposure to a suspicious package or substance, or required detailed medical expertise. SLT staff included a team of public health advisors to obtain initial case information, and at least two physicians, epidemiologists, or veterinarians. The SLT had an average of nine staff members (range 2–15) that was reduced in evenings and on weekends depending on the volume of calls being received. Whenever appropriate, calls were also referred from the SLT to state epidemiologists for more detailed follow-up.

Two Access databases (Microsoft Corp., Redmond, WA) were created to assist in documenting and tracking all incoming calls. A general database was intended to document every incoming call to the EOC telephone bank. For all incoming calls—call volume permitting—central telephone bank staff were instructed to record information on the date, topic, and type of caller on call response forms. SLT staff regularly collected these forms for manual data entry. Reports of call volume, call type, and call topic by day were then shared with EOC management and communications personnel to assist them with staffing decisions, publication of MMWR reports, and determination of educational needs.

Calls referred from the central telephone bank to the SLT were manually entered into a second, more detailed SLT tracking database. Information collected in this tracking database included demographic background of the patient, reporter information, and any reported symptoms or exposures. SLT staff were also asked to assign each referred call to a risk category to prioritize follow-up within the large volume of calls.

Telephone call data were exported from Access databases into Statistical Analysis Software (SAS Institute, Inc., Cary, NC). Distributions of call volume by date of call, type of caller, and topic of call were produced from the central EOC telephone bank data. Descriptive analyses of SLT tracking data were undertaken by type of caller, state of reported occurrence, triage classification (level of urgency), reported signs and symptoms, and nature of reported exposure. Data were analyzed during the peak period of call volume during the anthrax investigations (October 8 to November 11, 2001).

Results

EOC Telephone Bank Data

From October 8 to November 11, 2001, a total of 11,063 telephone calls were documented and responded to by EOC telephone bank staff. A topic of call was indicated for 4,178 (37.8%) of the calls. The most frequently mentioned topic was "questions about the availability of an anthrax vaccine" (2,438 [58.4%] of 4,178 calls), followed by "request for general bioterrorism information" (617 calls [14.8%]), "request for information about personal protective equipment" (501 calls [12.0%]), "general concerns about bioterrorism" (491 calls [11.8%]), and "request for information about smallpox" (400 calls [9.6%]).[1]

The type of caller was indicated on 6,845 (61.9%) of the 11,063 call forms. The most frequent types of callers included private citizens (3,712 [54.2%] of 6,845 calls), followed by physicians (1,846 calls [27.0%]), other federal or state employees (714 calls [10.4%]), and nonphysician health-care professionals (672 calls [9.8%]).[1] A greater percentage of calls from private citizens (42.5%) than from health professionals (32.1%) mentioned concerns about smallpox, bioterrorism, or requests for bioterrorism information. Health professionals (2.7%) were more likely than private citizens (0.7%) to ask questions about sample handling and processing.

Call volume increased to a peak of 858 calls received on October 16, 2001, shortly after the public announcement that a letter containing anthrax had been opened in Senator Tom Daschle's office (Figure 2). After that date, call volume to the EOC decreased each week. While the highly publicized nature of the bioterrorism-related events contributed to the large number of calls received by the EOC, day of the week was also an important determinant of call volume. Fewer calls were received on the weekends of October 13–14, 20–21, and 27–28 and November 3–4 and 10–11. During the period of data collection, the mean call volume to the EOC was 80 incoming calls per day on weekends and 411 incoming calls per day on weekdays. During weekdays, a lower call volume was also consistently observed on Mondays and Fridays. An average of 350 incoming calls per day were received on Mondays and Fridays and 450 incoming calls per day during Tuesday through Thursday. The proportion of calls received by topic of call and type of caller did not change in any meaningful way during this time (data not shown).

SLT Follow-Up Tracking Data

Of the 11,063 calls received by the EOC telephone bank, 882 (8.0%) were referred to the SLT for follow-up. Calls referred to the SLT came most commonly from physicians (256 calls [29.0%]), followed by private citizens (178 calls [20.1%]); state health department employees (99 calls [11.2%]); local government, law enforcement, or emergency personnel (99 calls [11.2%]); and nonphysician health-care workers (82 calls [9.3%]). The type of caller was not documented for 168 (19.0%) of the calls referred to the SLT.

The SLT staff provided follow-up on calls from 48 states, the District of Columbia, Puerto Rico, and Guam. Figure 3 presents the distribution of these calls by state of occurrence. While the distribution of calls by state was generally population based, a larger proportion of calls were received from states with increased press coverage of confirmed cases of anthrax and from Georgia, where CDC headquarters is

[1]Percentages do not add up to 100% as a call could include more than one topic (e.g., requests for information about more than one topic) or type of caller (e.g., caller is a physician who works at a state health department).

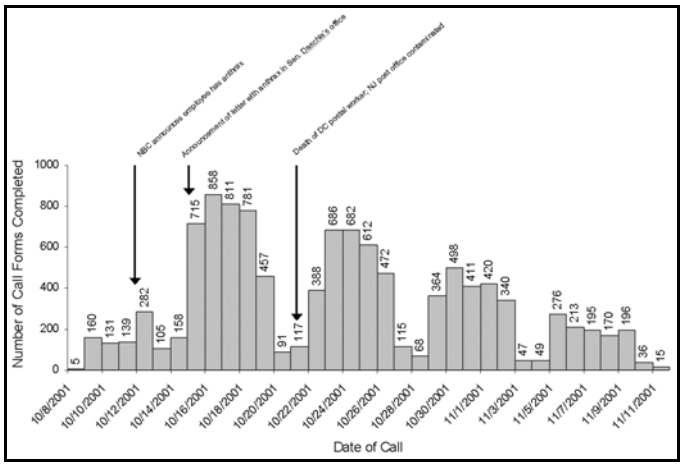

Figure 2. Telephone calls documented by staff of the Emergency Operations Center telephone bank, October 8 to November 11, 2001 (n = 11,063 call forms)

located. Forty-six percent of SLT follow-up activities pertained to reported occurrences in Washington, D.C., Georgia, New York, California, Maryland, and Pennsylvania (Figure 3). The proportion of calls received from private citizens or physicians did not vary by region of the country (data not shown).

Because SLT staff was limited to an average of nine members, a triage protocol to classify calls referred to the SLT by level of urgency was developed (Table 1). In 10.4% of the calls referred to the SLT for follow-up, a physician or health-care professional reported symptoms clinically compatible with anthrax in a person from a known high-risk group (postal workers, U.S. government officials, national press from contaminated facilities, or person with known contact with a contaminated facility) or in a person who reported exposure to a suspicious substance. An additional 15.2% of calls referred to the SLT included a report by a health-care professional of a person with clinically compatible symptoms but no reported high-risk status or possible source of exposure. Forty-four percent of all calls referred to the SLT mentioned exposure to a suspicious package or substance but did not include any report of clinically confirmed signs or symptoms. An additional 30.4% of the calls referred to the SLT included no mention of any reported exposures, signs, or symptoms (Table 1).

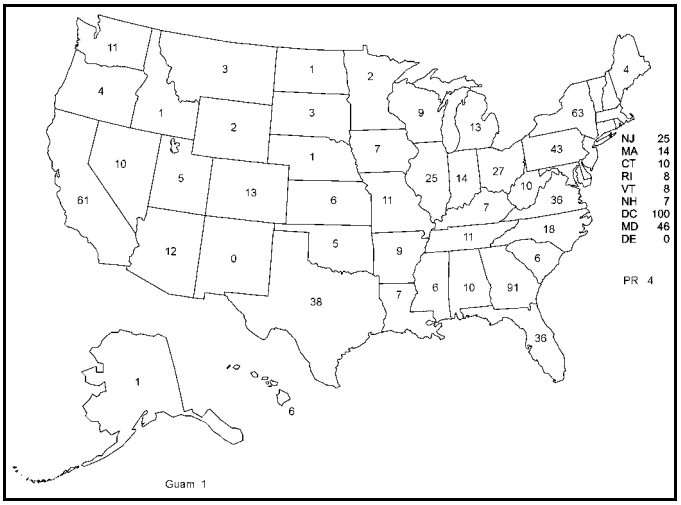

Figure 3. Distribution of telephone calls referred to the State Liaison Team, by state of occurrence, October 8 to November 11, 2001 (n = 882 calls)

Of the 181 calls referred to SLT that mentioned signs or symptoms clinically compatible with anthrax (classified as level A, B, C, or E in Table 1), fever or influenzalike symptoms were most commonly reported (57 calls [31%]). Other commonly reported signs and symptoms included skin lesions or eschars (48 calls [26.5%]), upper respiratory symptoms (47 calls [26.0%]), and skin rashes (19 calls [10.5%]). Fewer calls included mention of sore throats (15 calls [8.3%]), myalgia (15 calls [8.3%]), gastrointestinal problems (8 calls [4.4%]), lymphadenopathy (6 calls [3.3%]), chest pain (6 calls [3.3%]), and shortness of breath (4 calls [2.2%]).[1]

Four hundred eighty calls (54.4%) referred to the SLT included mention of exposure to a suspicious substance or package or direct contact with an environment known to be contaminated with *B. anthracis* (classified as level B, C, D, or F in Table 1). Over half of reported exposures included mention of contact with a "suspicious" powder or package (Table 2). However, <10% of reported exposures (47/480) included mention of any clinically confirmed signs or symptoms compatible with anthrax. As a result, standardized response protocols to address the handling of suspicious packages and powders and the receipt of mail through contaminated facilities were developed (5,7). This measure allowed second-tier triage staff to devote more time to calls involving clinically compatible cases from high-risk groups and SLT medical staff to remain on-call at off-site locations during evenings and weekends.

None of the calls referred to the SLT were confirmed to be reports of cases of anthrax. The confirmed cases of anthrax were identified by the CDC field specialty teams or through calls made to the CDC director.

Discussion

From October 8 to November 11, 2001, the EOC received 11,063 telephone calls pertaining to bioterrorism and referred 882 of these calls to the SLT for diagnostic evaluation, consultation, and coordination of follow-up activities. The volume of calls received during this time period demonstrated a considerable public need for guidance during this emergency.

Highly publicized incidents such as the opening of the letter in Sen. Daschle's office were likely catalysts for the observed increases in call volume. However, day-to-day patterns in the call volume to the EOC telephone bank suggest that at predictable times during the week emergency staff resources can be relaxed. During the data collection period, the mean call volume to the EOC was 80% lower on weekends than on weekdays. Within the working week, mean call volumes were 23% lower on Mondays and Fridays than during the rest of the work week. As many staff worked 12–20 hour

[1]Percentages do not add up to 100% because callers often reported more than one sign or symptom.

Table 1. Telephone calls referred to Emergency Operations Center State Liaison Team (SLT), by risk category, October 8 to November 11, 2001

Risk/urgency classification	Criteria	Frequency (N = 882)	Percent (%) of all calls referred to SLT
Level 1: A "Confirmed"	A. Clinically compatible[a] case -*and*- B. Isolation of *Bacillus anthracis* or two supportive lab results.	0[b]	0.0
B	A. Clinically compatible case -*and*- B. No isolation of *B. anthracis*, but one supportive lab result -*or*-epidemiologic link to confirmed exposure but no supportive lab results	2	0.2
C	A. Clinically compatible case –*and*- B. No epidemiologic link and no lab results -*and*- C. Known high-risk group: postal worker, U.S. government official, national press from contaminated facilities/or person with known contact with a contaminated facility -*or*-Ingestion of, inhalation of, or dermal contact with suspicious substance	45	5.1
D	A. No illness (or reports of symptoms that are clinically unconfirmed by a health professional) -*and*- B. Known direct exposure to environment confirmed to be contaminated with *B. anthracis*	45	5.1
E	A. Clinically compatible case –*and*- B. Not in high-risk group, -*and*- C. No lab results or epidemiologic link, -*and*- D. No known exposures to suspicious substance or packages	134	15.2
F	A. No illness (or reports of symptoms that are clinically unconfirmed by a health professional) -*and*- B. Not in high-risk group, -*and*- C. No lab results or epidemiologic link, -*and*- D. Ingestion of, inhalation of, or dermal contact with suspicious substance, or received mail directly from facility known to be contaminated during period of investigation.	388	44.0
G	A. No illness (or reports of symptoms that are clinically unconfirmed by a health professional) -*and*- B. Not in high-risk group, -*and*- C. No lab results or epidemiologic link, -*and*- D. No known exposure to suspicious powder or packages.	247	28.0
Unknown/ not classified	A. Unknown or call not related to anthrax	21	2.4

[a]Clinically compatible refers to physician or health professional report of any symptom thought to be related to inhalational, cutaneous, or gastrointestinal anthrax.
[b]Cases of anthrax confirmed during this time period were identified through active surveillance by CDC field epidemiology teams and not the Emergency Operations Center telephone bank.

days during the height of this emergency, allowing staff to remain "on-call" at off-site locations on days of predictably lower call volume may help maintain staff morale and stamina through long periods of emergency center operations.

The EOC implemented a tiered telephone call triage system designed to allow highly suspicious cases and exposures to be tracked more closely by field epidemiology and specialty teams (Figure 1). Using scripted responses to frequently asked questions, this system effectively screened out many calls involving general queries about anthrax vaccines, requests for bioterrorism information, and the use of personal protective equipment. This approach allowed the SLT at the second tier of the triage system to spend more time interpreting clinically confirmed symptoms and laboratory results, and monitoring possible exposures for further referral to appropriate specialized teams.

These findings, however, also indicate that many calls received by the SLT did not pertain to known high-risk situations. During the data collection period, nearly 75% of calls referred to the SLT did not include a report of any clinically confirmed signs or symptoms or any direct contact with an environment known to be contaminated with *B. anthracis*. Of these calls, nearly 60% mentioned contact with a suspicious powder or package, but included no report of illness. As a result, to maintain specificity in tracking high-risk cases, scripted responses were developed to questions regarding 1) contact with unknown substances, 2) the receipt of mail through a facility that had been contaminated with *B. anthracis*, and 3) the report of clinically unconfirmed signs or symptoms (5,7). We recommend further refinement of these response protocols for inclusion in the first tier of the triage system, along with additional training of telephone bank staff in the overall objectives and methods of triage during bioterrorism emergencies. These measures would substantially reduce the call volume burden on second-tier staff and decrease the chance that a high-risk situation would be overlooked during a similar bioterrorism event.

State health departments typically expect that CDC will direct local calls back to them unless they have previously

Table 2. Nature of reported exposure reported in telephone calls referred to the State Liaison Team, October 8 to November 11, 2001

Reported exposure	No.	Percent (%)
Received letter or package with suspicious powder	181	37.7
Visited location where *Bacillus anthracis* was isolated	102	21.3
Unspecified exposure to suspicious powder	81	16.9
Received mail from mail facility where *B. anthracis* was isolated	57	11.9
Received suspicious package without powder	20	4.2
Other	38	7.9
Unknown	1	0.1
Total	480[a]	100

[a]480 calls included a report of exposure.

been referred to CDC (9). As more than half the calls to the EOC were from private citizens, a larger number of calls should also have been redirected from the EOC to appropriate contact persons at the state level (with minimal data entry and analysis by CDC). Such referrals would have allowed the EOC staff more time to respond to questions from physicians or health departments. The extent to which state and local health departments were satisfied with the assistance received from the EOC also remains unknown. A survey of state and local personnel who contacted the EOC system would assist CDC staff with quality improvement of the triage system and provide additional insight into the state perspective of appropriate respective roles during periods of emergency response.

These data have several limitations. An unknown number of calls to the EOC telephone bank were undocumented as first-tier staff were unable to complete all telephone call response forms during peak periods of call intensity. These high call volumes periodically resulted in delays in information transfer between tiers of the telephone call triaging system. In addition, the manual completion of telephone response forms resulted in a substantial amount of missing data, as first and second-tier EOC staff often overlooked key data elements in their efforts to provide timely responses to public demands. Several coding classification schemes on the telephone response forms also require revision. For example, we were unable retrospectively to determine the number of law enforcement or emergency medical service personnel who called the central phone bank or whether callers from state health departments were medical or public relations personnel.

Telephone-based hotlines underestimate the true number of cases of a disease and are dependent on media reports and general public interest (10). However, a telephone bank at CDC during an outbreak of hantavirus pulmonary syndrome identified 38% of confirmed cases (10). Computerization of the EOC triage system, including required fields for date and topic of call and type of caller would allow for timely transfer and analysis of complete and accurate telephone call data and perhaps provide a similar layer of passive surveillance for emerging bioterrorism events. However, the maintenance of such a system would require additional technical expertise in database development, management, and analysis (11). Medical expertise in first-tier telephone bank staff will continue to be needed to assure the accurate entry of data into any automated system.

Our findings suggest that available on-site staff resources can be adjusted to predictable daily patterns of call volume to increase long-term effectiveness and stamina during emergency periods. While the first tier of the EOC telephone call triage system effectively addressed a substantial portion of all incoming public inquiries during this emergency, standardized health communication protocols that address contact with suspicious substances, handling of suspicious mail, and the clinical evaluation of suspected cases in the absence and presence of confirmed exposure should also be added to first-tier response activities in a computerized triage system. This standardization would allow for a more effective triage system for inquiries and more efficient focus for follow-up investigations by specialized epidemiologic teams.

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References

1. Centers for Disease Control and Prevention. Notice to readers: ongoing investigation of anthrax—Florida, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:877.
2. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
3. Centers for Disease Control and Prevention. Bioterrorism-related anthrax, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1008–10.
4. Bush LM, Abrams BH, Beall A, Johnson CC. Index case of fatal inhalational anthrax due to bioterrorism in the United States. N Engl J Med 2001;345:1607–10.
5. Centers for Disease Control and Prevention. CDC website: anthrax information. Accessed 4/24/2001. Available from: URL: http://www.bt.cdc.gov/Agent/Anthrax/AnthraxGen.asp
6. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and adverse events from antimicrobial prophylaxis. MMWR Morb Mortal Wkly Rep 2001;50:973–6.
7. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
8. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
9. Centers for Disease Control and Prevention. Website: Interim recommended notification procedures for local and state public health department leaders in the event of a bioterrorist incident. Accessed 7/14/2002. Available from: URL: http://www.bt.cdc.gov/emcontact/protocols.asp
10. Tappero JW, Khan AS, Pinner RW, Wenger JD, Graber JM, Armstrong LR, et al. Utility of emergency, telephone-based national surveillance for hantavirus pulmonary syndrome. JAMA 1996;275:398–400.
11. Stark C, Christie P, Marr AC. How to do it: Run an emergency hotline. BMJ 1994; 309:44–5.

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Coordinated Response to Reports of Possible Anthrax Contamination, Idaho, 2001

Leslie Tengelsen,* Richard Hudson,* Shana Barnes,† and Christine Hahn*

In 2001, the intentional release of anthrax spores in the eastern United States increased concern about exposure to anthrax nationwide, and residents of Idaho sought assistance. Response from state and local agencies was required, increasing the strain on epidemiologists, laboratorians, and communications personnel. In late 2001, Idaho's public health communications system handled 133 calls about suspicious powders. For each call, a multiagency bridge call was established, and participants (public health officials, epidemiologists, police, Federal Bureau of Investigation personnel, hazardous materials officials, and others) determined which samples would be tested by the state public health laboratory. A triage system for calls helped relieve the burden on public safety and health systems.

After the intentional spread of anthrax spores in 2001, states without anthrax cases were nonetheless affected by the outbreak. Idaho recorded a sharp rise in emergency calls, and the response requirements for traditional first responders, public health officials, laboratorians, and communications personnel increased. Before the outbreak, public health officials and first responders had little experience in jointly managing health-related issues. New response protocols and functional interagency relationships needed to be developed rapidly. Responders were faced with new scenarios and an increased call volume. In addition, safe handling protocols were needed for managing potential anthrax cases and handling clinical samples. The response and distribution of timely, accurate information between local, state, and federal public health partners, first responders, the health-care community, and the general public were crucial. Through this experience, procedures have been streamlined for a more effective response.

Notification and Initial Response to Possible Anthrax Exposures

Anecdotal information suggests that all states had to respond to public inquiries about powdery substances found in the mail or public areas. Despite being removed geographically from anthrax cases and contaminated sites, Idaho was no exception. The state uses a centralized State Emergency Medical Services Communications Center (StateComm), which receives emergency calls in areas that lack 911 services and provides the emergency communication system for and between all state agencies. This center was established in 1974 through a Robert Wood Johnson Foundation grant to enhance rural Emergency Management System communications services but has expanded over the last 10 years to include public health inquiries. StateComm, which is part of the Idaho Department of Health and Welfare, operates 22 remote mountaintop transmitter sites connected by microwave links to a central location. StateComm staff dispatch regional hazardous materials (hazmat) teams, page public health officials, and provide bridge call services; up to 48 ports are available for a single bridge call.

From August 1 to October 7, 2001, StateComm received 73 routine hazmat calls and no biohazard calls, which was a typical calling pattern for the hotline (Figure). However, from October 8 to December 31, 2001, StateComm received 53 routine hazmat calls and 133 biohazard calls; all biohazard calls were related to suspicious powders. Most of the biohazard calls were made by local law enforcement, who were on-scene incident commanders following state hazmat response protocols during powder investigations. StateComm staff then convened emergency bridge calls for each biohazard call and used state hazmat protocols to determine who should participate in the call. Public health, law enforcement (including Federal Bureau of Investigation [FBI]), hazmat, and other officials routinely participated in these calls and discussed how to respond to possible anthrax exposures. All powder-related incidents were treated as potential criminal acts, and all samples were maintained as evidence to ensure a standardized response. For each call, participants asked the incident commander if a written threat was present and who was the apparent target. If an

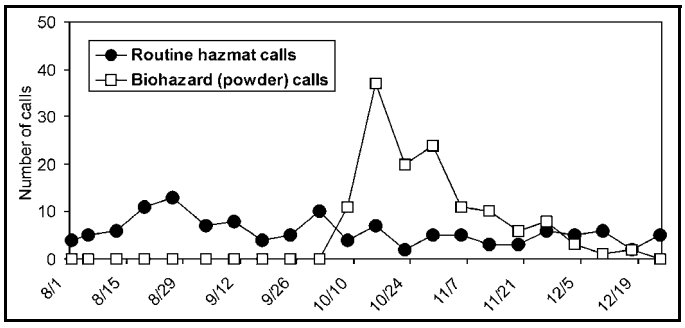

Figure. Calls received by the Idaho State Communications Center from August 1, 2001 to December 31, 2001, are shown by category: routine hazardous materials calls and biohazard (suspicious powder calls).

*Idaho Department of Health and Welfare, Boise, Idaho, USA; and †State Emergency Management Systems, Meridian, Idaho, USA

envelope or package had a return address, the on-scene incident commander contacted the sender to verify that he or she sent the item and to identify its contents. The threat level was then assessed based on suspicious package guidelines (1) and other requirements listed previously.

During the first days of calls, emergency bridge call participants agreed that no samples would be sent to the state public health laboratory for testing until approved by call participants. The state public health laboratory was notified when samples were routed to them. Persons who may have been exposed to anthrax were informed by the on-scene incident commander that results would be available within 48 h, that antibiotics were not recommended pending test results, and that they were free to consult with their medical provider. With this protocol, only 50 (37.6%) of the biohazard calls yielded items for testing by the state Bureau of Laboratories. All test results from the laboratory were reported directly to StateComm, which then notified the on-scene incident commander of the results.

Laboratory Testing

The state public health laboratory in Boise is the only laboratory in Idaho that accepts environmental samples for anthrax testing. All 50 suspect exposure incidents, as determined by a multiagency bridge call, were given numeric identifiers by StateComm and linked to powder samples being routed to the state public health laboratory. Transportation across Idaho was facilitated by a state police escort to maintain the chain of custody. The state public health laboratory established an on-site chain of custody protocol with local, state, or FBI law enforcement officials before the microbiologic evaluation of any item. Thirty incidents yielded postmarked items for testing (letters, envelopes, and packages). Seven incidents yielded swabs or vials of powder for testing. Miscellaneous objects received for testing included clothing, a mailbox, a handheld vacuum, a pillbox, a toy, a dollar bill, and a crate. Three of the letters contained threats, which necessitated FBI involvement. Objects with possible contamination were evaluated for spores with the spore stain (Malachite green) by wet-mount-phase microscopy and were cultured for *Bacillus anthracis* under modified biosafety level-3 conditions (2). Although some objects contained *Bacillus* species, all were negative for *B. anthracis* by gamma-phage testing. A turnaround time of 24 h or less was generally maintained for presumptive determinations, and StateComm was alerted immediately of presumptive negative test results. A final culture-negative determination was made 48 h after receipt of the sample. Laboratorians followed protocols provided by the Centers for Disease Control and Prevention Laboratory Response Network. That the staff of three microbiologists was not enough to handle the dramatic increase in workload was soon evident. Therefore, 11 additional laboratorians were trained in test procedures, and the staff was grouped into two-person teams to provide around-the-clock coverage. Facility biosecurity was increased, with locked entries, a sign-in desk, and guest badges.

Health-Care Outreach

Idaho has documented rare, naturally occurring cases of anthrax. The last human case of cutaneous anthrax occurred in 1964, and the last documented animal case occurred in a cow in 1984. The state epidemiology staff developed two sets of public health guidelines for health-care providers, which included information about the epidemiology of naturally occurring anthrax in Idaho, the features of the current outbreak of anthrax (1,3,4), and the possible risk to postal workers. The guidelines also included information about the availability of in-state testing and state and local public health contacts. These guidelines were faxed to the seven district health departments, which in turn faxed them to health-care providers, emergency rooms, and infection control practitioners, following the Health Alert Network system protocols. The guidelines were also placed on the state health department Web site and faxed or mailed to providers, media, and citizens who requested anthrax information.

During October 2001, local physicians contacted the state epidemiology office for assistance in evaluating and treating 12 possible anthrax cases: 11 persons with possible inhalational anthrax (6 [54.5%] were postal workers) and 1 person with possible cutaneous anthrax. All human samples were negative for anthrax. To better understand what syndromic signs and symptoms created suspicion in health-care providers, we reviewed 9 of 12 suspected anthrax cases (Table). Information was gathered and compared with the first 10 confirmed inhalational anthrax cases in the United States (5). Occupational risk played a key role in suspicion of pulmonary anthrax infection; however, the symptoms of the suspected anthrax cases varied greatly from those of confirmed anthrax cases. These findings were included in follow-up information sent to health-care providers.

Discussion

Reviewing the problems encountered in Idaho and how they were addressed may improve the public health response

Table. Clinical comparison of confirmed versus suspected inhalational anthrax cases

Characteristics	Confirmed cases[a] n=10 (%)	Idaho suspected cases, n=9[b] (%)
Postal worker or mail sorter	8 (80)	6/11[c] (54)
Fever/chills	10 (100)	2 (22)
Fatigue/malaise	10 (100)	8 (89)
Sweats	7 (70)	2 (22)
Cough	9 (90)	7 (78)
Nausea or vomiting	9 (90)	2 (22)
Dyspnea	8 (80)	3 (33)
Rhinorrhea	1 (10)	4 (44)

[a]Multistate cases confirmed by Centers for Disease Control and Prevention.
[b]Nine of the suspected inhalational cases had charts available for review.
[c]11 suspected inhalational anthrax cases.

in all states. A centralized communications center is critical for reducing the impact of a large-scale outbreak on a public health emergency response system and for providing timely and consistent response to citizens. In Idaho, the preexisting communications system enabled communication between multiple agencies. The rapid development of triage protocols is important for consistent response to a crisis. While basic response protocols must be outlined for each event, a rapid mechanism for protocol development and agreement by participants must be part of any flexible response plan. Local health departments should be included in biohazard response protocols to minimize confusion during the management and follow-up of each public health event. Initially, StateComm calls included only state health department officials because local health officials did not carry pagers. Local health officials in Idaho are now equipped with pagers and are part of the response protocol. Immediate reporting of laboratory test results to a central communications center reduces the burden on laboratory staff. The volume of callers seeking results was decreased because health and law officials were aware that results could be obtained from the communications center directly. Extra effort and time attempting to reach first responders, citizens, and health officials with test results were eliminated in this manner.

In responding to suspected bioterrorist events, treating each event as a possible crime requires cooperation and planning. Transport of samples by law enforcement required cooperation with multiple county and state law enforcement officials. Alternative transportation plans would have been useful in Idaho should a local law enforcement agency have refused to transport a specimen. In addition, the establishment of a proper chain of custody and proper packaging procedures would have allowed more streamlined processing of samples for both laboratory safety and chain-of-custody requirements. Education of state communications personnel in communicable disease topics, such as anthrax, is required if communications personnel are used to initiate and coordinate response protocols to biohazard events. A basic understanding of the terminology and the general principles of epidemiologic response would minimize the chance that a potentially serious situation is overlooked. Trust between the decision-makers in multiple local, state and federal agencies is essential for coordinating responses effectively. In small states, fewer people are usually involved in each response, and the same participants tend to be on each call, simplifying coordination. Planning meetings with other responding agencies are essential in order to establish protocols and to foster trust.

States with small, rural populations often have fewer resources to deal with the increasing stress on their emergency response systems. Despite the lack of anthrax infections in the western United States during the fall of 2001, citizens in Idaho were fearful of being exposed to anthrax, and the public health emergency response system was tested. A well-coordinated response was required from agencies with little experience in working together. Idaho was fortunate to have a statewide communications network in place; however, even with this response system, modifications were required to ensure smooth relationships between first responders and public health officials.

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References

1. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
2. Chapin KC, Murray PR. Strains. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yoken RH, editors. Manual of Clinical Microbiology. 7th ed. Washington: American Society for Microbiology; 1999. p. 1678.
3. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–93.
4. Centers for Disease Control and Prevention. Notice to readers: interim recommendations for protecting workers from exposure to *Bacillus anthracis* in work sites in which mail is handled or processed. MMWR Morb Mortal Wkly Rep 2001;50:961
5. Jernigan JA, Stephens DA, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.

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Laboratory Response to Anthrax Bioterrorism, New York City, 2001

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In October 2001, the greater New York City Metropolitan Area was the scene of a bioterrorism attack. The scale of the public response to this attack was not foreseen and threatened to overwhelm the Bioterrorism Response Laboratory's (BTRL) ability to process and test environmental samples. In a joint effort with the Centers for Disease Control and Prevention and the cooperation of the Department of Defense, a massive effort was launched to maintain and sustain the laboratory response and return test results in a timely fashion. This effort was largely successful. The development and expansion of the facility are described, as are the special needs of a BTRL. The establishment of a Laboratory Bioterrorism Command Center and protocols for sample intake, processing, reporting, security, testing, staffing, and quality control are also described.

Laboratories across the United States have been preparing for the past 5 years for the possibility of civilian populations being the target of bioterrorism (1). The New York City (NYC) Department of Health (DOH) laboratory response plans for bioterrorism changed forever after October 12, 2001, with the knowledge that letters laden with *Bacillus anthracis* spores had been sent through the U. S. Postal Service (2). The original conception of the laboratory's role in bioterrorism response was not yet fully validated, nor was the need for extensive environmental testing fully appreciated or anticipated. The number of personnel with specialized training was another key factor.

The most probable scenario envisioned a sharp increase in hospital admissions caused by one of the recognized bioterrorism agents (3). By the time the symptoms and bioterrorism agent were diagnosed, the disease was likely to be well established within the local population. Thus, laboratory response would center primarily on human clinical sampling. The scope of required environmental sampling was not fully anticipated and was generally considered to be secondary to the original epidemiologic investigation. Such samples would predominantly consist of evidence obtained from the putative source of the exposure.

Although this was the operational scenario, the actual laboratory workload during this event was evenly divided between environmental and clinical samples. However, the amount of labor and materials associated with processing environmental samples for analysis far exceeded that of the clinical samples.

Background: Laboratory Structure before October 2001

Before October 12, the NYC Public Health Laboratory (PHL) processed one or two suspected bioterrorism environmental samples per month, utilizing a small Biosafety Level 2 (BSL-2) room with two dedicated personnel. In the year before the attack, the PHL received approximately 10 samples, all of which were hoaxes. The laboratory was set up according to Centers for Disease Control and Prevention (CDC) protocols, and staff were trained by CDC on methods for isolating and identifying bioterrorism agents.

The bioterrorism laboratory consisted of a 400–square-foot area designed at BSL-2+ as described in Biosafety in Microbi-

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[2]The New York City Anthrax Investigation Working Group consisted of Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (CDC), Fort Collins: May Chu, David Dennis, Kathleen Julian, Anthony A. Marfin, and Lyle Petersen; CDC, Atlanta: Mary Brandt, Richard Kanwal, Kristy Kubota, Els Mathieu, Steve Ostroff, John Painter, Dejana Selenic, Allison Stock, Linda Weigel, and William Wong; New York City Department of Health: Neal Cohen, Laura Mascuch, Denis Nash, Sarah Perl, and Don Weiss; New York City Public Health Laboratories: Alice Agasan, Jay Amurao, Josephine Atamian, Debra Cook, Erica DeBernardo, Adeleh Ebrahimzadeh, Philomena Fleckenstein, Anne Marie Incalicchio, John Kornblum, Ed Lee, William Oleszko, Lynn Paynter, Alexander Ramon, Chiminyan Sathyakumar, Harold Smalls, George Williams, Marie T. Wong, and Ben Y. Zhao.

ological and Biomedical Laboratories (4). Entrance to the laboratory was controlled by proximity card access and monitored 24 hours a day by video cameras. The space contained a biosafety cabinet, a fluorescence/phase-contrast microscope, incubators, freezers and refrigerators, a Wallach/Perkin Elmer Victor Time Resolved Fluorescence instrument (The Perkin-Elmer Corp., Norwalk, CN), computers, and necessary laboratory supplies. This configuration provided a comfortable and controlled access space for sample preparation and analysis. Because of the low sample volume, each sample was treated uniquely, and a generalized method for handling numbers of environmental specimens was not considered necessary.

Before October 12, all specimens submitted to NYC BTRL were tested for four priority bioterrorism agents: *B. anthracis* (anthrax), *Francisella tularensis* (tularemia), *Yersinia pestis* (plague), and *Brucella* species (brucellosis). Protocols defined and validated by CDC were used to isolate and identify these agents (5). All specimens tested during that time were culture negative for the four priority bioterrorism agents according to the validated protocols.

October 12: First Letter Tested Positive

Before *B. anthracis* was identified in letter C from media outlet 1**,** two other letters (A and B) were received and tested by BTRL. Letter A came from media outlet 1, and letter B came from media outlet 2. Letters A and B were tested for the four priority bioterrorism agents and were negative. At the time, the negative result for letter A was somewhat surprising because the patient diagnosed with cutaneous anthrax was employed by media outlet 1. When letter C later arrived at BTRL, it was tested and found to contain a powdery substance that was positively identified as spores of *B. anthracis.* The discrepancy involving the positive results of letters A and C was soon resolved when it was determined that letter C was actually received before letter A at media outlet 1 but was inadvertently placed in a corporate "hate-mail" file and was thus recovered after letter A.

A number of important events took place almost simultaneously after letter C tested positive for *B. anthracis*: 1) the BTRL was contaminated with *B. anthracis* spores during the sampling process and three BTRL laboratory employees were exposed; 2) the news media and the U.S. Attorney General broadcast a message to Americans asking them to report all suspicious mail to their local law enforcement authorities (6); and 3) as a result of this increased attention, the sample volume surged and did not abate for another 6 weeks.

These events worked synergistically to complicate NYC DOH's ability to contend with bioterrorism testing on the scale needed during this crisis. At this time, CDC contacted NYC DOH to offer support and aid. On learning of the situation developing in NYC and the events surrounding the contamination of BTRL, including exposure of employees, the PHL, in conjunction with CDC, instituted several important policies: 1) A Bioterrorism Response Laboratory Command Center was established at PHL to direct and coordinate all bioterrorism laboratory activities and communications; 2) A secure and separate entryway was set up so bioterrorism specimens could enter the PHL building without jeopardizing the safety of PHL building personnel; 3) A separate specimen-receiving area containing a decontamination site was established, and all specimens were double bagged and externally decontaminated (sprayed with a bleach solution) before being brought to the testing laboratory for analysis; 4) All environmental bioterrorism specimens were tested by using strict and secure BSL-3 containment and BSL-3 protocols; 5) BTRL personnel exposed in the contaminated laboratory were treated with ciprofloxacin HCl; 6) Extensive infection control and environmental monitoring procedures were set up throughout the PHL building to monitor for *B. anthracis* spores; 7) Security was extensively increased throughout the building's interior and exterior; 8) During the transition to the new BSL-3 testing facility, samples received for bioterrorism testing were shipped to offsite level C laboratories for analysis; 9) A dedicated database was developed for accepting and tracking bioterrorism specimens and testing results; and 10) CDC and NYC DOH requested a Department of Defense (DOD) Microbiology Response Team to assist with rapid testing of bioterrorism specimens.

After the initial evaluation, the NYC PHL facility was configured to operate 24 hours a day, accepting, processing, and testing samples. Additional laboratory space was identified, consisting of three separate areas for handling and testing bioterrorism samples (two polymerase chain reaction [PCR] units and an enzyme immunoassay [EIA] rapid screening unit). The BTRL coordinator was also appointed to work in conjunction with CDC and DOD teams. Staffs from other units were also redeployed to further assist in the bioterrorism response effort.

Post–October 12: The Bioterrorism Response Laboratory

Within days of the initial event on October 12, all the essential elements of BTRL were in place. Table 1 describes the transition before and after October 12. Both the types of laboratory activities and their scale changed dramatically. The sample volume increased approximately 3,000 times for both environmental and clinical testing. Not surprisingly, the number of laboratories and ancillary spaces BTRL required increased almost twentyfold, and 25 times more personnel than originally envisioned staffed these additional areas. New instrumentation (i.e., the PCR rapid assays) was brought into BTRL to attempt to process the sample volume more quickly. To supply this dramatic surge, six tons of equipment and supplies was needed. The scale of the operation and the tracking needs threatened to overwhelm the support staff, and a hastily constructed but workable database system was put into place.

This sample volume surge was expected to be specimens of human origin (clinical specimens); the need for large-scale environmental sampling and testing had not been anticipated. The clinical laboratories experienced exponential increases in volume but had enough latent capacity to handle the increased

Table 1. Comparison of the New York City Bioterrorism Response Laboratory requirements before and after October 12, 2001[a,b]

	Before October 12, 2001	After the surge of specimens
Specimen load	1 every 2–3 months	2,700 nasal swabs/2 weeks 3,200 environmental specimens/2 months
Laboratory space	One room	10 laboratories 3 evidence rooms 4 support areas Command center (suite of offices) Separate storage area for supplies
Staff	2 people rotating on call schedule	>75[c]
Technology	Basic microbiology capabilities γ phage DFA	Rapid PCR assays with conventional basic microbiology capabilities
Supplies	General laboratory supplies	6 tons flown to NYC from CDC
Miscellaneous	No database 1 stand-alone computer	Clinical database Environmental database 30 computers linking all areas of the building

[a]When the first letter tested positive for spores of *Bacillus anthracis* was received.
[b]PCR, polymerase chain reaction; DFA, direct fluorescent antibody assay; CDC, Centers for Disease Control and Prevention; NYC, New York City.
[c]From the NYC Public Health Laboratory, CDC, NYC Department of Health, and Department of Defense.

workload. Increases in coverage and overtime, plus additional reagents, sufficed to contain the testing volume within manageable limits. Clinical sample processing and tracking were not adversely affected, but environmental sampling was severely hampered. The original testing laboratory was never designed to handle more than perhaps a few samples per day. On the first day of the surge, the laboratory received 34 samples that were considered high priority (Figure 1). Figure 2 shows the flow of a sample as it enters the BTRL. The laboratory can be divided into three main functional entities: 1) a receiving area, which contains a decontamination site in processing area and a secured temporary storage facility; 2) two sampling areas (one each of BSL-2 and BSL-3), containing facilities to unwrap and examine environmental samples and retrieve samples for further analysis (BSL-3), clinical microbiology laboratories, and the PCR laboratories; and 3) locked and guarded storage for samples that had completed the testing protocol and were ready for subsequent distribution as waste, returnable property, or evidence.

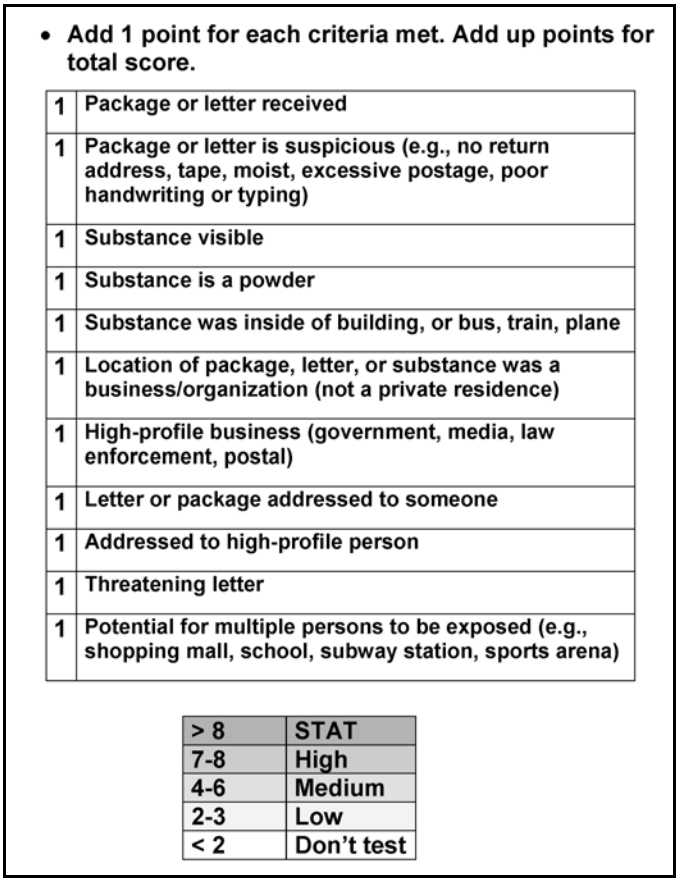

Figure 1. Depiction of the algorithm used to determine the priority of items received for testing at the New York City Bioterrorism Response Laboratory. One of the salient features of the surge was the broad array of items that the laboratory received for testing. Many items contained innocuous powdery substances that are now known to be unrelated to the attack, yet prudent practices required that they be ruled out. The laboratory needed to identify which items were the most urgent and place them first and used this algorithm and other triage methods to prioritize the samples. Samples with 8 out of 11 points or greater were deemed STAT for "highest priority for laboratory testing" and received preferential treatment. Most samples fell into a middle category and were processed in order based on time received.

Bioterrorism Response Laboratory: Units, Operation, and Staffing

Samples were tracked through the system by a specially designed database that reflected the testing status of the sample and its final report status. A large portion of the database was devoted to description and demographics (Figure 3). Table 2 shows a section of a typical spreadsheet.

All environmental samples entered the building through the designated bioterrorism intake area. The main function of this area was to provide decontamination, documentation, and security. Samples would be accepted only from designated first responders and law enforcement personnel. Although standard protocols now ensure that the samples brought in for laboratory testing are not externally contaminated with a bioterrorism agent (7), as a prudent preventive measure the outer

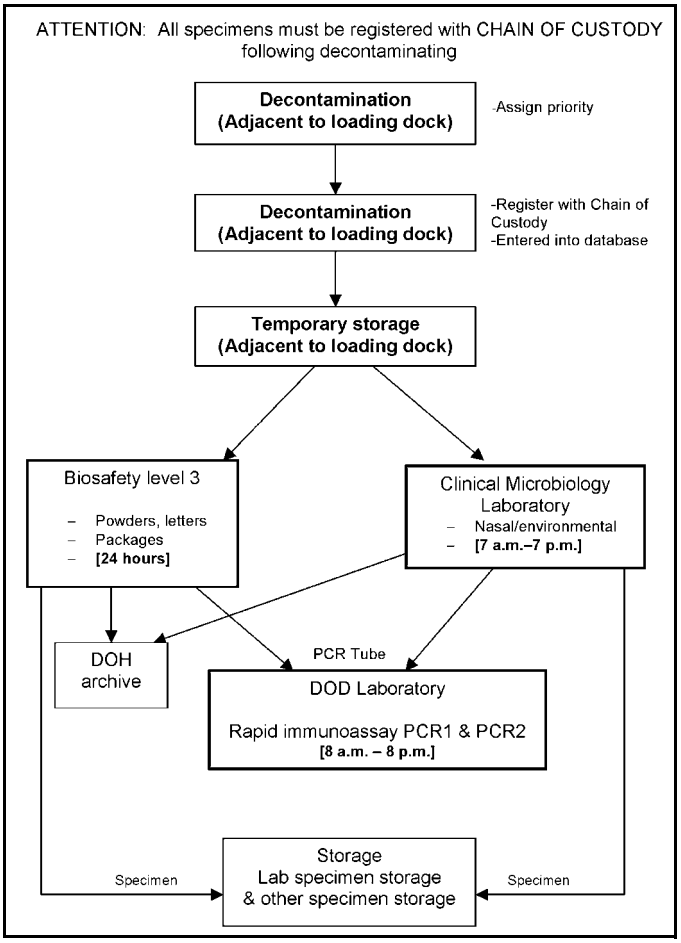

Figure 2. Diagrammatic tracking of an environmental sample through the various units and laboratories as it was processed and tested for anthrax at the New York City Bioterrorism Response Laboratory. The first level of the diagram corresponds to the first floor or the sample intake area. Samples were moved via an elevator to the upper floors of the facility, where they were processed and tested. The final destination of all samples was the storage area. Storage was also a locked and guarded forensic evidence room, and samples released from this area after testing negative for *Bacillus anthracis* were released to the New York Police Department for criminal investigation, return, or disposal.

packaging still needed to be decontaminated in the intake area. A breach in any procedure could compromise the laboratory.

Chain-of-custody documentation was maintained in the intake unit as well as initial entry into the database. All packages came with a test request/manifest document with the data entered and manually maintained at the intake area. Security (provided by NYC DOH Police Department) were present in the area continuously. After passing through decontamination and receiving, packages were held in a nearby temporary storage area until requested by the sampling or testing laboratories.

Analytical Units

The analytical laboratory was composed of four units: 1) high-containment examination area (BSL-3), where all environmental samples suspected of containing dispersible powders were examined and sampled for further testing; 2) BSL-2 laboratory, for environmental swabs; 3) clinical microbiology, for receiving clinical swabs and analyzing tissue samples; and 4) rapid testing, where the EIA and PCR-based systems were employed, designed to quickly yield preliminary data in advance of the classical microbiology final report.

Storage

After a sample was tested, it was sequestered in a safe, secure area. Samples testing positive for a bioterrorism agent were stored in a specifically designated, locked storage area separate from the negative samples. All negative samples once recorded were handed over to NYPD, where the items were screened for evidentiary purposes. Items not considered evidence were autoclaved and returned to their owners, if valuable. Otherwise, they were discarded. NYPD maintained a log of all transactions and signed off the final disposition on the chain-of-custody form completing the case.

Laboratory Operation

After a sample passed through the intake area, it either entered the BSL-3 testing area or proceeded as a clinical sample or swab directly into the clinical microbiology unit. Swabs

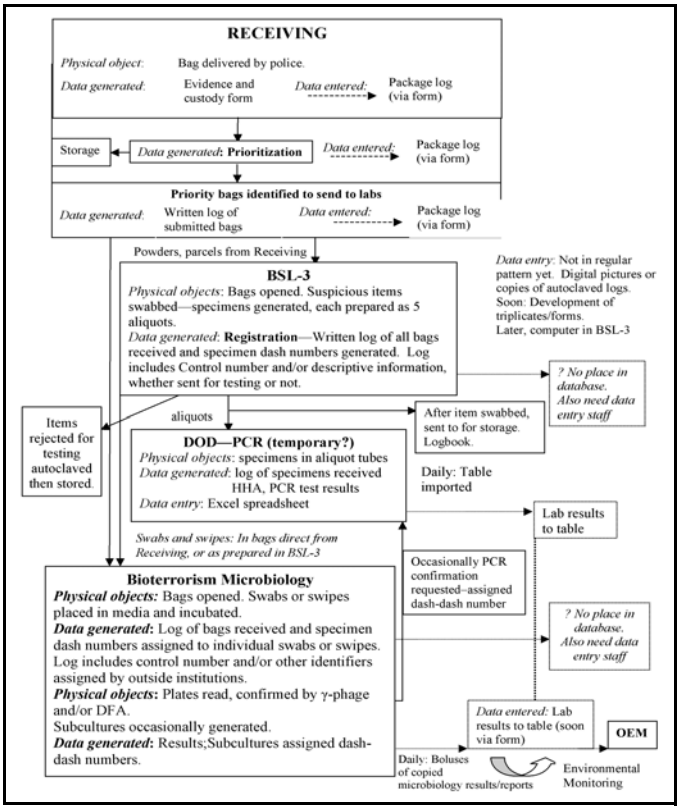

Figure 3. Depiction of the data flow at the New York City Bioterrorism Response Laboratory adopted soon after the surge of isolates after the bioterrorism attack. An access database was developed, and a number of demographic fields and test results were identified and entered. Data retrieved from the Biosafety Level 3 (BSL-3) laboratory after suspicious packages were opened had to be input into the database; the original documentation was modified if any additional information was identified. An attempt was made to monitor all transactions occurring to the sample, which began to make the system unwieldy. The database was modified numerous times and recently was entirely replaced. Most of the comments, such as "no place in database" have been corrected. PCR, polymerase chain reaction; DOD, Department of Defense; HHA, handheld analysis; DFA, direct fluorescent-antibody assay; OEM, Office of Emergency Management.

Table 2. A sample section of the data table generated by the tracking system diagramed in Figure 3[a,b]

Site address[c]	Pick-up date	Intake date	Item description	Testing location	Urgency	Comments	Swab taken?
FBI	10/9/2001	10/9/2001	Envelope (Westchester County)	NYCPHL			No
Hospital A	10/10/2001	10/10/2001	Blood culture	NYCPHL	Stat		No
Hospital B	10/8/2001	10/10/2001	Request for bacterial culture identification	NYCPHL	Stat		No
FBI	10/10/2001	10/10/2001	Petri dish	NYCPHL			No
NYPD		10/11/2001	One express-mail envelope sealed in plastic, addressed to United Nations	NYCPHL	High		No
FBI	10/11/2001	10/11/2001	Plastic bag with white powder; business card.	Wadsworth	Low	not enough info	No
FBI	10/11/2001	10/11/2001	Plastic bag containing one envelope with white powder.	Wadsworth	Low	not enough info	No

[a]From left to right are fields for responder or site of response, site address, date of pick-up, date of intake, bag contents, location of testing, comments, priority, swab taken (yes, no), and patient (if clinical sample). This database allowed the managers to check the progress of sampling and keep track of the "who, what, where, and when" of the samples.
[b] FBI, Federal Bureau of Investigation; NYCPHL, New York City Public Health Laboratory; NYPD, New York City Police Department; Stat, highest priority for laboratory testing.
[c]Masked for security purposes.

taken from letters, powders, objects, clothes, and other items in the high containment BSL-3 area were plated directly on sheep blood agar (SBA) or transferred onto brain heart infusion broth (BHIB) and incubated there. Another set of samples was taken for rapid testing. These PCR samples were brought out of the containment area and sent to the rapid testing units in separate sample bags decontaminated with a recommended hypochlorite solution (4).

On completion of sampling, the specimen was removed from the biosafety cabinet and taken to the evidence storage area. This procedure posed a problem since it is recommended that items leaving the BSL-3 area be fully decontaminated. Since steam sterilization or chemical decontamination might destroy valuable evidence, the finished items were placed into sterile biohazard bags that remained uncontaminated on the outside. This newly packaged sample was then removed to the evidence storage area.

Testing Protocols and Reporting Algorithm

All testing protocols were adapted from established protocols (8). In short, samples were analyzed by using a rapid screening assay (PCR) to provide preliminary information to health-care providers and law enforcement. However, final disposition of samples was only made after exhaustive identification according to recommended microbiology protocols.

Figure 4 outlines the workflow through the analytical units. Clinical samples were generally directly plated onto SBA. The environmental samples often were simultaneously transferred into BHIB and heat shocked to kill nonsporulating organisms and enrich for *B. anthracis* spores. A sample was reported as positive only if it had all the following phenotypes: nonmotile; penicillin sensitive; γ-phage positive; and positive by both cell wall and capsule direct fluorescent-antibody assay. Extensive environmental monitoring was performed on the reports before they were released. All negative clinical reports were compiled into a manifest and sent to the Mayor's Office of Emergency Management, where they were distributed to the appropriate parties.

At the peak of the surge, BTRL was testing 60–100 samples per 24-hour shift. Each sample required, at a minimum, duplicate PCR and an SBA culture. Any growth required the

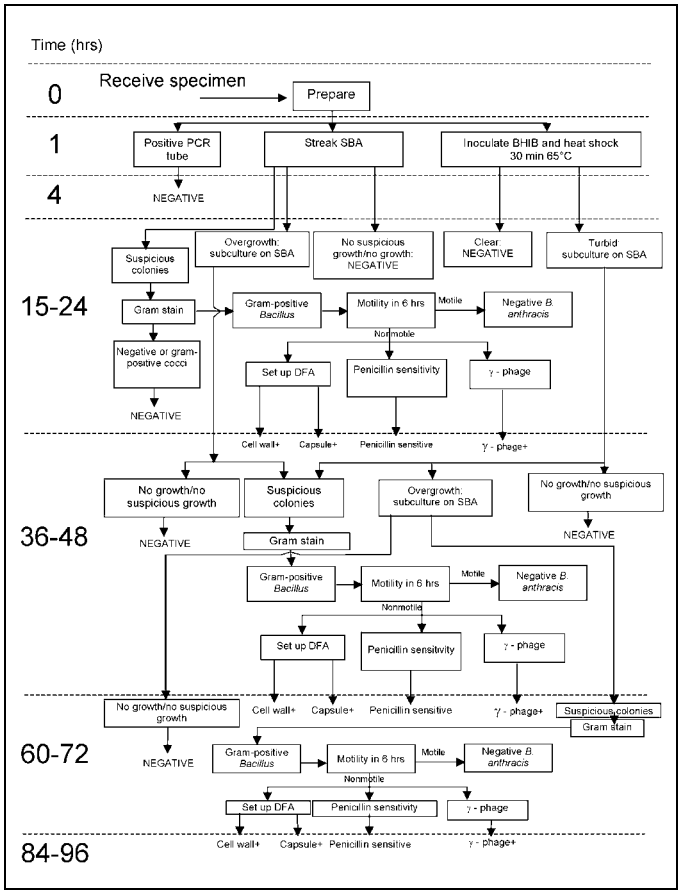

Figure 4. Chart tracking the time needed to report the status of a sample brought in for classical *Bacillus anthracis* testing at the New York Bioterrorism Response Laboratory. Negative samples with no suspicious growth could be reported in 24 hours. However, any samples with growth required some degree of subplating or culturing in brain heart infusion broth (BHIB), were heat shocked, and then tested. Reporting of final results on samples could take 3–4 days. SBA, sheep blood agar; CW, cell wall; CAP, capsule; DFA, direct fluorescent-antibody assay; PCR, polymerase chain reaction; Ph, phage; +, positive.

phenotypic testing described above. Most samples were also transferred to BHIB; growth in that medium required phenotypic analysis. The average sample, even if it resulted in a negative finding, required at least 14 separate testing procedures to determine its status.

Processing specimens sampled in the BSL-3 required 30–60 minutes and limited the flow to the microbiology laboratories. Despite any effort to speed testing through the microbiology laboratories, the limiting factor remained the maximal BSL-3 throughput of approximately 40 samples per 24 hours.

Biosafety and Environmental Monitoring

Normal operations within a clinical microbiology laboratory require routine infection control and quarterly environmental monitoring (9). Because of the experience of October 12 when one laboratory was contaminated, hypervigilance was required to prevent the possibility of further contamination. Our concerns were not only for safety but also for the integrity of the testing process, as stray contamination could seriously mitigate the reliability of the laboratories results.

We instituted a schedule of infection control environmental monitoring. Typical areas that would signal contamination such as door handles, laboratory benches, and hoods, in addition to exposed skin of technical personnel, were routinely sampled each day. Approximately 70 data points were routinely sampled from the various bioterrorism units around the facility, including the intake area, elevators to the BSL-3, the BSL-3 (all three shifts), microbiology laboratories, PCR laboratories, and all personnel associated with operations. Additional areas sampled frequently were the storage room and various corridors in the facility.

Laboratory Staffing

With minor variations, the PHL had a fully functional BTRL running 24 hours a day, 7 days a week within the first week. Approximately 75 personnel from DOH, CDC, DOD, and other organizations were split evenly between two shifts per day. Scheduling was further complicated because DOH personnel had to be borrowed from other testing units and could not be dedicated to the bioterrorism effort alone.

Staffing during the surge consisted of DOH personnel along with the CDC emergency team. The DOD Microbiology Rapid Response Team that supported testing in the microbiology and BSL-3 sampling unit filled a number of slots. The DOD was also completely responsible for the rapid testing units.

A unique aspect of the staffing requirements for BTRL was the need for extensive security. The DOH Police Department carried out this function. Officers were present in the intake area and guarded the sensitive testing and storage areas around the clock. They were responsible for maintaining the chain of custody and for initial intake of information after the first response units brought samples to the laboratory. In addition, officers increased all aspects of security for the building with extensive identification (ID) checks, closed circuit television surveillance, and maintenance of ID cards. Essentially, the DOH Police Department continuously monitored all personnel movement in the facility.

Physical Security

Physical security concerns became paramount because samples brought to the BTRL were also potential criminal evidence and therefore required special precautions (e.g., chain of custody, locked or guarded storage areas) to protect their integrity. To accomplish these security goals, the DOH Police Department augmented laboratory security by increased background investigation of personnel, extensive implementation of physical security procedures, and oversight of laboratory accessions and evidence containment. The DOH Police Department investigated unusual work practices, breaches of confidentiality, and safety issues with an eye to possible lapses in security.

Physical security was enhanced by the use of ID cards, restricted area badges, and a sign-in logbook. Only one entrance was open to the public, while another entrance was designated for bioterrorism sample accession. Card access was instituted for all sensitive areas such as the testing laboratories and the evidence room. This system allowed for tracking of users and limiting such use to specific personnel at specific times. All card and badge access was tracked. All entrances, elevators, emergency exits, and sensitive laboratories were monitored continuously by closed-circuit television, and all transactions were recorded.

The use of biological, chemical, or radiologic materials with the intent of causing injury or death is a crime, and the instrument used and swabs or specimens obtained from the crime scene are potentially evidence (10). The DOH Police Department maintained responsibility for accepting and storing proper evidence to maintain its integrity as it was transferred from law enforcement into the laboratory for testing. Custody containment, which ensured the integrity of the evidence for prosecution, was also maintained by the DOH Police Department.

Conclusion

The events of September 11, 2001, placed New York City on high alert immediately (11). On the heels of this tragedy, the City became the target of a bioterrorism attack (12). NYC DOH, as part of the city's emergency response network, was extensively involved with the mitigation of both these catastrophes. The laboratory had recent experience in public health emergencies such as the West Nile virus outbreak (13) and the 1999 bottled-water scare.

Although PHL had chain-of-custody experience through its Toxicology and Environmental Laboratories and outbreak testing during the West Nile outbreak, nothing could have prepared the laboratory for the events of October 2001. Nevertheless, staff outfitted the laboratory within days to accept, test, report, store, and return data or evidence from literally thousands of environmental and clinical samples tested for anthrax.

In the months after the crisis, BTRL still receives about five suspicious samples per week. Samples are now routinely tested for the four priority agents, and plans have been finalized for dedicated laboratory space designed by using the lessons learned from October 2001.

Nevertheless, before October 2001, we thought we were prepared to confront an event on the scale of this bioterrorism attack. An important lesson from this experience is that, despite all additional precautions and enhancements made to the laboratory and the response network, another attack, if and when it occurs, will present further surprises. While the laboratory has now institutionalized weapons of mass destruction testing to be performed as part of routine surveillance (e.g., testing of drinking water), potential means and targets for future attacks cannot be perfectly forecast. Vigilance and continued emphasis on flexibility, creativity, and the ability to rapidly expand our response, as needed, to bioterrorism events and the surprises they present will determine our effectiveness and ultimate success.

We appreciate the efforts and support of the Surgeon General and their staffs of the U.S. Air Force and Navy.

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References

1. Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, et al. Anthrax as a biological weapon: medical and public health Management. JAMA 1999;281:1735–45.
2. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
3. Franz DR, Jahrling PB, Friedlander AM, McClain DJ, Hoover DL, Bryne WR, et al. Clinical recognition and management of patients exposed to biological warfare agents. JAMA 1997;278:399–411.
4. Richmond JY, McKinney RW, editors. Biosafety in microbiological and biomedical laboratories. HHS pub. no. CDC 93-8395, 3rd edition. Washington: U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; 1993.
5. Centers for Disease Control and Prevention. Public health emergency preparedness and response: agents of bioterrorism. CDC:PHEPR 2002. Available from: URL: http://www.bt.cdc.gov/labissues/index.asp
6. Underwood A, Springen K. Newsweek: Anthrax: a spreading scare: the medical facts. PR Newswire 2001 Oct 14. Available from: URL: http://www.findarticles.com
7. New York City Public Health Laboratories. Draft bioterrorism response laboratory protocols. New York: New York City Department of Health; 2002.
8. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yoken RH. Manual of clinical microbiology. 7th edition. Washington: American Society for Microbiology; 1999.
9. New York City Department of Health. Exposure control plans. New York: Department of Health; 2001.
10. United States Code: Title 18, Section 2332(a): Added Pub. L. 103-322, Title VI, Section 60023(a) (Sept. 13, 1994, amended Oct. 21, 1998) Threatening to use a weapon of mass destruction against federal property.
11. Centers for Disease Control and Prevention. New York City Department of Health response to terrorist attack, September 11, 2001. MMWR Morb Mortal Wkly Rep 2001;50:821–2.
12. Centers for Disease Control and Prevention. Investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
13. 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.

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Specific, Sensitive, and Quantitative Enzyme-Linked Immunosorbent Assay for Human Immunoglobulin G Antibodies to Anthrax Toxin Protective Antigen

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The bioterrorism-associated human anthrax epidemic in the fall of 2001 highlighted the need for a sensitive, reproducible, and specific laboratory test for the confirmatory diagnosis of human anthrax. The Centers for Disease Control and Prevention developed, optimized, and rapidly qualified an enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) antibodies to *Bacillus anthracis* protective antigen (PA) in human serum. The qualified ELISA had a minimum detection limit of 0.06 µg/mL, a reliable lower limit of detection of 0.09 µg/mL, and a lower limit of quantification in undiluted serum specimens of 3.0 µg/mL anti-PA IgG. The diagnostic sensitivity of the assay was 97.8%, and the diagnostic specificity was 97.6%. A competitive inhibition anti-PA IgG ELISA was also developed to enhance diagnostic specificity to 100%. The anti-PA ELISAs proved valuable for the confirmation of cases of cutaneous and inhalational anthrax and evaluation of patients in whom the diagnosis of anthrax was being considered.

Naturally occurring anthrax is a zoonotic disease of herbivores, with low-level sporadic infection of humans. Since 1950, human anthrax in the United States was confined to those occupationally at risk, with only 235 confirmed cases, mostly cutaneous, reported from 1955 to 2002 (1–3). The occurrence of human anthrax in the country and the public perception of the disease changed dramatically in the fall of 2001, with the first successful bioterrorist anthrax attack on the U.S. civilian population. This event necessitated the simultaneous development and application of qualified laboratory assays—including serologic assays—to evaluate patients suspected of having anthrax.

The major obstacle to serologic analysis of human anthrax has been the lack of assay standardization. Variations in antigen preparation and purity, assay methods, and endpoint determination between laboratories and the absence of a suitable standard reference serum compound this problem. The Centers for Disease Control and Prevention (CDC) had, before the attacks, instituted the development of anthrax serologic assays—particularly enzyme-linked immunosorbent assays (ELISAs)—for use in anthrax vaccine studies in humans and to provide a standard human reference serum. In response to the anthrax emergency of 2001, we report the accelerated development and qualification of a quantitative ELISA for detection of anti-protective antigen (PA) specific immunoglobulin (Ig) G in human serum and the development of a competitive inhibition assay to enhance diagnostic specificity. The assays were applied to diagnosis of cutaneous and inhalational anthrax to evaluate serologic responses in persons considered at risk from anthrax spore exposure and enhance anthrax serologic tests with standardized techniques for distribution to public health and clinical laboratories.

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Methods

Antigen Preparation

Recombinant anthrax toxin protective antigen (rPA) with an amino acid sequence concurring with that from the *Bacillus anthracis* V770-NP1-R anthrax vaccine strain was obtained

from the National Institute of Craniofacial and Dental Research, National Institutes of Health, Bethesda, MD. Antigen was stored frozen at –80°C in small aliquots (10–100 µL, 4.75 mg/mL) in 5 mM Hepes, pH 7.3. Antigen was expressed from the attenuated asporogenous host *B. anthracis* BH445 and purified to homogeneity as described (4).

Human Serum for Determination of Diagnostic Specificity and Sensitivity

To determine the background level of anti-PA ELISA reactivity in a cross-section of the U.S. population, a panel of 238 control sera from healthy adult persons was assembled from the CDC Occupation Health Service and the National Health and Nutrition Examination Survey (NHANES, CDC) serum collections. Donors were selected on the basis of having no known exposure to *B. anthracis* or anthrax and no known history of anthrax vaccination. In addition, a panel of 277 sera was assembled from persons with clinically confirmed non-anthrax-related illnesses (acute hepatitis A, acute hepatitis B, influenza A and B, brucellosis, staphylococcal toxic-shock syndrome, group A streptococcal infections, legionellosis, *Chlamydia pneumoniae* infection, and *Mycoplasma pneumoniae* infection) and from children and adults who had received non-anthrax-related vaccines (trivalent influenza, hepatitis B, tetanus toxoid, and botulinum toxoid). To determine assay sensitivity, an additional panel of 68 sera from persons who had received anthrax vaccine adsorbed (AVA) and 19 control sera from nonvaccinees was obtained. All sera were tested in duplicate without heat inactivation.

Human Standard Serum Preparation

The anti-AVA standard human reference serum, AVR414, was prepared by plasmapheresis of healthy adult CDC volunteers who had received at least four subcutaneous injections of Anthrax Vaccine Adsorbed (AVA, BioPort Corp., Lansing, MI) with the licensed regimen (0, 2, and 4 weeks; 6, 12, and 18 months; and yearly boosters). Plasmapheresis and serum conversion were done at the Emory Transfusion Medicine Program, Emory University School of Medicine (Atlanta, GA) and the Scientific Resource Program at CDC, respectively. Plasmapheresis was done by the TPE DUAL- NEEDLE procedure with the COBE Spectra Apheresis System (Gambro BCT, Inc., Blood Component Technology, Lakewood, CO) and following the manufacturer's procedure manual (Manual #701900–000 1999/1). Each plasma unit was clotted with sterile glass microbeads (B. Braun Instruments, Burlingame, CA) and suspended in 1.5 M $CaCl_2$–2.0 M ε-amino-caproic acid. All units were allowed to clot overnight at room temperature and were then centrifuged at 2,200 x *g* at 4°C for 15 min. The serum from each unit was stored in a 500-mL sterile plastic container. The level of residual anticoagulants was not measured. The total IgG concentration of the serum pool was determined by radial immunodiffusion and nephelometry, with the U.S. National Reference Preparation for Specific Human Serum Proteins (CDC) as a standard (5). Anti-PA specific IgG mass value assignment to the standard serum was done by differential adsorption, homologous enzyme-linked immunoassay (EIA), and heterologous ELISA (Semenova VA, et al., manuscript in preparation), with U.S. Food and Drug Administration (FDA) 1983 *Haemophilus influenzae* type b (Hib) reference serum (6).

ELISA Procedure

Polyoxyethylene sorbitol monolaurate (Tween 20) was purchased from BioRad Laboratories (Hercules, CA). Skim milk powder was obtained from Difco/Becton Dickinson (Atlanta, GA). Horseradish peroxidase (HRPO)–conjugated mouse anti-human IgG (affinity purified, γ-chain specific monoclonal clone HP6043) was obtained from Hybridoma Reagent Laboratories (Baldwin, MD). Peroxidase substrate 2,2′-azino-di(3-ethyl-benzthiazoline-6-sulfonate) (ABTS), hydrogen peroxide (H_2O_2), and peroxidase stop solution were obtained from Kirkegaard & Perry Laboratories (KPL, Gaithersburg, MD). All other laboratory reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified. Sterile, Type I endotoxin-free water was used for all ELISA procedures.

Immulon II-HB flat-bottom 96-well microtiter plates (Thermo Labsystems, Franklin, MA), were coated for 16 hrs at +4°C with 100 µL/well of rPA at a concentration of 2.0 µg/mL in 0.01 M phosphate-buffered saline (PBS), pH 7.4 (Life Technologies, Gaithersburg, MD). Plates were stored at +4°C without blocking and used within 7 days of preparation. Antigen-coated plates were then washed three times (ELX405 microplate washer, BioTek Instruments Inc., Winooski, VT) with PBS containing 0.1% Tween 20 and blotted dry by inversion on clean paper towels. Control and serum antibodies were tested without a separate blocking step. Serum standards and sera for testing were prepared at the appropriate dilutions in PBS containing 5% skim milk and 0.5% Tween 20, pH 7.4. The human standard reference serum and test sera were serially diluted twofold in the plate in the same buffer solution. The minimum dilution of test serum was 1/50. Three positive control sera from three separate donors and one negative control serum were each used at single dilution factors selected to give a range of optical density (OD) values across the standard reference curve. The final volume in all wells was 100 µL.

Test and standard sera were incubated in a humidified chamber (covered tray) for 60 min at 37°C, and the plates were then washed three times with PBS containing 0.1% Tween 20. Bound anti-PA IgG was then detected by using HRPO-conjugated mouse anti-human IgG Fc PAN monoclonal HP6043 diluted in PBS containing 5% skim milk and 0.5% Tween 20 (100 µL/well), and plates were incubated in a humidified chamber (covered tray) for 60 min at 37°C. Plates were again washed three times with PBS containing 0.1% Tween 20, and bound conjugate was detected colorimetrically by using ABTS/H_2O_2 substrate (100 µL/well). Color development was over 30 min (±5 min) and was stopped by addition of 100 µL of Peroxidase Stop Solution (KPL) to all wells of the test

plates. OD values were read within 30 min of addition of the stop solution with a MRX Revelation microtiter plate reader (Thermo Labsystems, Franklin, MA) at a wavelength of 410 nm with a 610-nm reference filter. Data were analyzed by using a four-parameter (4-PL) logistic-log curve fitting model with ELISA for Windows software (7). A calibration factor for the standard reference serum was used to determine the concentration of anti-PA IgG in micrograms per milliliter of serum (μg/mL).

Competitive Inhibition ELISA

To enhance specificity, a supplementary rPA competitive inhibition ELISA (CI-ELISA) was developed based on the qualified anti-PA IgG ELISA. The CI-ELISA was a direct extension of the standard ELISA procedure with the following modifications. The anti-PA antibody concentrations of the test sera were first determined by using the standard ELISA. Only sera with a minimum reactivity level of 10 μg/mL anti-PA antibody were suitable for evaluation in the CI-ELISA. The 10-μg/mL threshold was determined empirically as the minimum level for which a reduction in ELISA reactivity could be assigned with statistical significance. A concentration of 50 μg rPA/500 μL diluted sample was chosen as the absorbing concentration after a preliminary study with ranges between 0 and 200 μg/mL (8). Test sera were then diluted to a concentration calculated to provide an OD value of approximately 1.0, based on their reactivity in the standard anti-PA ELISA. A 1-mL volume of each diluted serum was prepared and divided into two aliquots of equal volume. To one of these aliquots, rPA was added to a final concentration of 100 μg/mL. Both tubes were capped tightly and mixed by inversion for 16–18 hrs at +4°C. After this incubation, the tubes were centrifuged at 4°C for 10 min at 8,000 x *g* to remove precipitated materials. Test sera were incubated in the presence and absence of an excess of rPA in solution before analysis in the standard ELISA.

The supernatants were used without further dilution in the standard ELISA described above. Based on defined sera from anthrax vaccine recipients and confirmed clinical cases, a ≥85% suppression of reactivity in the competitive ELISA was identified as the threshold to discriminate between true positives and false positives.

Accuracy, Precision, Limits of Quantification, and Goodness of Fit

Accuracy describes the exactness of the assay to measure a known, true value of anti-PA IgG and to measure it repeatedly. In this study, accuracy was determined by repeated analysis of a positive control human anti-AVA antiserum for which differential absorption and heterologous ELISA had determined the anti-PA IgG concentration. Accuracy is expressed as the percent error between the assay-determined value and the assigned value for that serum. A percent error of ≤20% is an acceptable level of accuracy for an enzyme immunoassay (9). Precision, a measure of the degree of repeatability of an assay under normal operating conditions, is expressed as the coefficient of variation of the concentrations calculated for the standard reference curve dilutions within a single assay plate (intraassay precision) and between different assay plates (interassay precision) determined over time and controlling for different operators. Acceptable levels of intraassay and interassay precision are 10% and 20%, respectively (9), and these can be used to define the range of the assay and the upper and lower limits of quantification. The range of the assay is the interval between the upper and lower levels of antibody (inclusive) that have been demonstrated to be determined with these levels of precision and accuracy.

The "goodness of fit" of the assay is, for comparative purposes, an indication of how closely the data points of the reference serum standard curve fit the 4-PL model. Goodness of fit is expressed as the regression coefficient (R^2) of the standard curve. An R^2 value that approaches unity is indicative of a good fit for the data to the curve (9).

Limits of Detection of the Anti-PA IgG ELISA

The 4-PL function was used to model the characteristic curve for the standards data. These data exhibit a sigmoidal shape when plotted on an OD-$\log_{10}$ dilution scale. The 4-PL function fits these data with a high degree of accuracy and extends the range of the assay, thus providing a more precise measurement of antibody concentration for patient sera (10). The lowest concentration of analyte (anti-PA IgG) that can be detected with a specific degree of probability in a diluted serum sample is defined as the minimum detectable concentration (MDC). The lowest concentration of analyte that has a high probability of producing a response significantly greater than the response at zero concentration of analyte is defined as the reliable detection limit (RDL). The MDC and RDL of the anti-PA IgG ELISA were derived from a 4-PL fit applied to the AVR414 standard reference serum (9). The MDC is the concentration of anti-PA antibody corresponding to the interpolated intersection of the lower asymptote of the upper 95% confidence interval (95% CI) with the 4-PL fit of the standards data. The RDL is the concentration of anti-PA antibody corresponding to the interpolated intersection of the upper 95% CI asymptote with the lower-95% CI of the standards data. The MDC and RDL are thus both derived from the 95% CIs of the standard curve. They are distinct and statistically robust measurements of the lower limits of detection of the assay; the RDL is the more conservative of the two. An illustration of the relationship of MDC and RDL to the standard curve is shown (Figure).

The reactivity threshold (Figure) is used to categorize a serum as reactive or nonreactive and to determine the diagnostic sensitivity (DSN) and diagnostic specificity (DSP) of the assay. The reactivity threshold of this assay was determined from the frequency distribution (11) of $\log_{10}$-transformed OD values from a panel of sera from humans with non-anthrax-related clinical infections (554 observations) and a panel of control human sera (476 observations). The reactivity threshold was determined as the upper 95% CI of the frequency

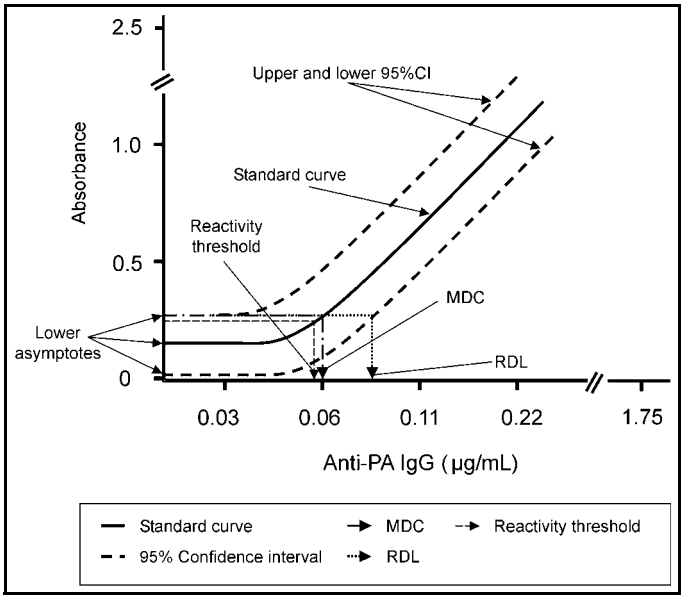

Figure. Graphic representation of minimum detectable concentration (MDC), reliable detection limit (RDL), and reactivity threshold. The MDC is the concentration of anti-protective antigen antibody (anti-PA) corresponding to the interpolated intersection of the lower asymptote of the upper 95% confidence limit with the 4-parameter logistic log fit of the standard curve data. The RDL is the concentration of anti-PA antibody corresponding to the interpolated intersection of the lower asymptote of the upper 95% confidence limit with the lower 95% confidence limit of the standard's data. The reactivity threshold was determined as the upper 95% confidence limit of the frequency distribution from $\log_{10}$-transformed optical density (OD) values of control human sera tested at 1/50 dilution. This OD value was converted to an anti-PA immunoglobulin (Ig) G concentration by using the standard curve calibration factor. Where this calculated value is below the MDC of the assay, the MDC was selected as the default reactivity threshold.

distribution from $\log_{10}$-transformed OD values of control human sera tested at 1/50 dilution. This OD value was converted to an anti-PA IgG concentration by using the standard curve calibration factor. Where this calculated value is below the MDC of the assay, the MDC becomes the default reactivity threshold. Ideally, the MDC, RDL, and reactivity threshold will all fall within the limits of quantification as defined above.

ELISA Diagnostic Sensitivity and Specificity

The DSP and DSN of the anti-PA IgG ELISA were determined. The quantitative test results were categorized into reactive or nonreactive by application of the reactivity threshold. The DSP of the assay was calculated as [TN/(TN+FP)], where TN = true negatives and FP = false positives. The DSN of the assay was calculated as [TP/(TP+FN)], where TP = true positives and FN = false negatives. Initially, serum specimens from clinical anthrax cases were insufficient to be useful in determining the DSN of the anti-PA IgG ELISA. Thus, the DSN was calculated by using sera from a cohort of anthrax vaccine recipients who had received a minimum of four subcutaneous injections of AVA. The DSN of the assay was reevaluated at the end of the anthrax emergency, when a greater number of specimens from clinical cases had accumulated.

Human Sera from Patients with Confirmed or Suspected Anthrax or Exposure to *B. anthracis* Spores

The qualified anti-PA ELISA was applied to sera from persons with confirmed or suspected anthrax cases and from persons exposed to *B. anthracis* spores. Blood was collected in serum separation tubes and allowed to clot; the serum was then separated from clotted cells by low-speed centrifugation. Serum was shipped to CDC with a unique identification number. All clinical serum samples were blinded to the laboratory team and tested in duplicate. All ELISA-reactive sera were tested a minimum of twice. The CI-ELISA was applied to single serum specimens with a reactivity of $\geq$10 µg/mL and when persons' paired sera indicated reactivity in the absence of changing anti-PA antibody concentrations over time. A $\geq$4-fold rise over the calculated value for the acute serum or the assay reactivity threshold was used to define seroconversion.

Anti-PA IgG concentrations in test sera were calculated by interpolation to the standard reference calibration curve by using the ELISA for Windows Software Version 1.0 (7); anti-PA IgG concentrations were expressed in micrograms per milliliter of the original serum sample. For results to be reportable, the assay was required to meet a set of quality control acceptance criteria. For an acceptable level of precision, the mean anti-PA IgG concentrations for three separate quality control sera were required to calculate within 3 standard deviations (SDs) of their assigned mean concentrations; at least two of the mean anti-PA IgG concentrations for these sera were required to be within 2 SDs of their respective assigned mean values. Assay plates were also evaluated for parallelism between the standard curve and the test samples (12).

Results

Performance Characteristics of the Anti-PA IgG ELISA

Feasibility, standardization, and performance of an ELISA to detect IgG antibodies against the PA of *B. anthracis* were completed during the anthrax epidemic of fall 2001. A human serum pool (AVR203) for which the anti-PA IgG concentration had been determined empirically was used to establish the accuracy of the ELISA. The percent error between the assigned value and the assay-determined value was 6.5%, as determined from independent analyses by three individual operators. These data are indicative of an acceptable level of accuracy for this type of assay (9). The performance characteristics of the AVR414 standard curve and of three positive quality control sera selected from humans vaccinated with AVA were used to determine the precision (repeatability) of the anti-PA ELISA. The positive quality control sera were tested in duplicate at single dilutions selected to represent high, medium, and low OD regions of the reference serum standard curve. The percent error was 15.6% for quality control serum #1 (n=55 tests), 20.2% for quality control serum #2 (n=92 tests), and 12.5% for quality control serum #3 (n=93 tests); the

average percent error from the three sera was 16.2%. The precision within a single assay plate, as expressed by the intraassay coefficient of variation of the AVR414 standard curve, was 8.5%, and the interassay precision was 17.0%. These values are within the accepted values of 10% and 20% for intraassay and interassay precision, respectively (11), and are indicative of a high level of precision for this type of assay (9). The goodness of fit (mean R^2) for the AVR414 standard curve calculated over 54 runs was 0.99. On the basis of the determinations of accuracy and precision given above, the range of calculable concentrations from the AVA414 standard curve is 0.06–1.7 μg/mL of anti-PA IgG with an MDC and an RDL of 0.06 μg/mL and 0.09 μg/mL, respectively (n=54).

Limits of Quantification and Reactivity Threshold

The limits of quantification are the lowest and highest concentrations of analyte that can be measured with a fixed degree of precision. The fixed degree of precision for this assay has been selected as a coefficient of variation (%CV) of 20% for the calibrated antibody concentration of the reference standard curve. For the anti-PA ELISA, the %CV for the calibrated antibody concentration is 20% for all of the standards data, indicating that the full extent of the AVR414 standard curve encompassing the MDC and RDL can be used in calculating an anti-PA IgG concentration for unknown sera and determining the reactivity threshold. The reactivity threshold for this assay was determined from the frequency distribution of $\log_{10}$-transformed OD values from non-anthrax-related human control sera. The geometric means for the OD values from 277 sera from humans with non-anthrax-related clinical infections (544 observations) and 238 (476 observations) human control sera were 0.059 (95% CI 0.018 to 0.253) and 0.050 (95% CI 0.021 to 0.138), respectively. The higher upper confidence limit of 0.253 was used for calculation of reactivity threshold. This corresponds to an anti-PA IgG concentration of 0.056 μg/mL, indicating that the lower asymptote of the standard curve is within the 95% CI of the control sera tested at the lowest dilution (1/50) used in this assay (Figure). Because the upper confidence limit of the control sera is less than the MDC (0.06 μg/mL) of the assay, the MDC of a 1/50 diluted serum becomes the default reactivity threshold and corresponds to an anti-PA IgG concentration of 3.0 μg/mL in an undiluted serum sample. By the same logic, a more conservative determination of reactivity threshold could be derived from the RDL (0.09 μg/mL) corresponding to a concentration of 4.5 μg/mL in an undiluted serum sample. However, using the MDC to derive the reactivity threshold maximizes the sensitivity of the assay without compromising specificity; thus, the reactivity threshold of 3.0 μg/mL was selected as the lower limit of quantification.

DSN and DSP

The reactivity threshold was used to categorize sera as reactive or nonreactive and then to determine the DSN and DSP of the assay. The reactivity threshold was determined empirically, avoiding assumptions on the antibody response rate following exposure to anthrax toxin PA whether by vaccination or clinical infection. The lack of counts in the false-positive cells (Table) for vaccinee and clinical anthrax sera and the lack of counts in the false-negative cells for normal and non-anthrax infection sera can be qualified on both their known exposure to PA and their reactivity in the anti-PA IgG ELISA. Because only a few serum specimens from clinically positive anthrax cases were available at the start of this emergency response, the DSN of the anti-PA IgG ELISA was initially determined by using 68 sera from a cohort of sera donated by AVA vaccinees. For this sample cohort, the numbers of true positives and false negatives were 67 and 1, respectively. The DSN of the assay under these conditions is therefore 98.5% (Table). When the control sera from the same sample set of donors were used, the numbers of true negatives and false positives were 15 and 4, respectively, suggesting a diagnostic specificity of 78.9% for determining whether

Table. Calculation of diagnostic sensitivity and diagnostic specificity by using cohorts of known vaccination or infection status[a]

Serum test group	AVA vaccinees	Non-Vaccinees	NHANES controls	Non-anthrax infections	Clinical anthrax sera[b]	Total sera of known infection and vaccination status
True positives[c]	67	0	0	0	15	82
True negatives[d]	0	15	228	260	0	503
False positives[e]	0	4	10	17	0	31
False negatives[f]	1	0	0	0	1	2
Total	68	19	238	277	16	618
Diagnostic specificity	n/a	78.9%	95.7%	93.8%	n/a	94.2%
Diagnostic sensitivity	98.5%	n/a	n/a	n/a	93.7%	97.6%

[a]AVA, anthrax vaccine adsorbed; NHANES, National Health and Nutrition Examination Survey; n/a, not applicable.
[b]Clinical anthrax sera were obtained from donors that met the Centers for Disease Control and Prevention case definition for confirmed cutaneous and inhalational anthrax. Patients were classified as either reactive or nonreactive.
[c]Sera are considered true positives if they were obtained from clinically confirmed anthrax cases or from donors with a documented history of anthrax vaccination.
[d]True-negative sera were selected on the basis of having no known exposure to *Bacillus anthracis* infection and no known anthrax vaccination.
[e]False-positive sera are defined as sera which reacted (>3.0 μg/mL) in the anti-protective antigen immunoglobulin G enzyme-linked immunosorbent assay but for which there is no history of clinical anthrax or anthrax vaccination.
[f]False-negative sera are defined as sera from donors who are documented as vaccine recipients or had clinically confirmed anthrax.

ELISA reactivity is due to an exposure to anthrax toxin PA (Table).

However, separate analyses of two further serum cohorts (sera from clinical infections other than anthrax and sera from NHANES negative controls) returned DSP values of 93.8% and 95.7%, respectively (Table). Combined analysis of all sera of known infection or known vaccination status, including sera from the 21 confirmed clinical anthrax cases, indicated an overall DSN of 97.8% and consequently a 2.2% frequency of potential false positives (Table).

Discussion

The focus of this report is to describe the qualification and performance characteristics of an ELISA for anti-PA IgG antibodies and enhancement of its specificity by using a second-stage CI-ELISA. The application of these assays to the analysis of the antibody response following anthrax infection (Quinn CP et al., manuscript in preparation) and for serologic surveillance from clinical anthrax cases will be reported in detail elsewhere (13,14). Historically, if not identified and treated early, systemic anthrax in humans was invariably fatal. As a consequence, serologic assays have not featured prominently in the diagnosis of clinical anthrax and in some reports have been considered unreliable for early identification of the disease or for establishing a retrospective diagnosis (15). Serologic assays for anthrax have primarily been applied for the evaluation of immune responses to anthrax vaccines, in epidemiologic investigations of the disease in animals, and in confirmatory diagnosis of the various manifestations of anthrax in humans (16–18). A useful adjunct to serologic analysis of anthrax infection is the Anthraxin test (19), which elicits a localized delayed-type hypersensitivity reaction to intradermal injection of a complex uncharacterized extract from attenuated vegetative *B. anthracis* cells or edema fluid from *B. anthracis*–infected animals. Anthraxin has been reported to be very accurate for retrospective verification of anthrax in humans (20), to be applicable for up to 30 years after infection (15), and to have a qualitative positive correlation with anti-PA antibodies in the sera from human clinical anthrax cases as detected by ELISA (21). Anthraxin is not, however, approved by FDA as a diagnostic reagent in the United States.

For nearly 4 decades, anthrax serologic studies depended on the Ouchterlony agar gel diffusion test (16), which in turn replaced complement fixation tests and in vivo passive protection and neutralization tests (22). The development of an indirect (passive) microhemagglutination test (23) was the next major progression in anthrax serologic testing. Based on the agglutination by serum antibodies of sheep erythrocytes sensitized with partially purified culture supernatants containing anthrax toxin PA (Factor II) (24), this test provided greater sensitivity and speed than the agar gel diffusion technique. The microhemagglutination assay, however, was laborious to set up because antigen-coated erythrocytes had only a short shelf life, and variation in erythrocyte batches compromised reproducibility (25).

The microhemagglutination assay was replaced by the more sensitive and reproducible ELISA system, which has been applied in various formats including the immobilized antigen (direct) assay (25), an immobilized anti-PA antibody (antigen-capture) assay (26), and a competition ELISA (27), with, where reported, varying degrees of specificity and sensitivity (17,18,28). Turnbull et al. (29) described using ELISA to confirm anthrax in humans and demonstrated that recipients of the licensed AVA could be distinguished from persons with natural infections on the basis of their lack of reactivity to the anthrax toxin lethal factor protein. Sirisanthana et al. (17) in a serologic study of anthrax in northern Thailand and Harrison et al. (18) in a serologic study of anthrax in Paraguay described use of both ELISA and Western blot to retrospectively evaluate seroconversion in cutaneous and oral-oropharyngeal anthrax. In both studies, separate ELISAs were applied for the detection of anti-toxin and anti-capsule antibody responses, and Western blot was used to enhance the specificity of serologic diagnosis (17,18). Turnbull et al. (16) reported the detection of anti-PA antibodies in humans and animals in the Etosha National Park and also concluded that there is a residual antibody level in these populations in an area where the disease is endemic. However, the specificity and sensitivity of the ELISA used in that study were not reported, and the frequency of anti-PA antibodies in bacteriologically confirmed clinical cases was a maximum of 71% (16). The prevalence of true positive anti-PA antibody reactivity in the general human population therefore remains unknown, although our study of 515 non-anthrax-related control sera suggests that it is probably <7%.

The anti-PA ELISA developed and qualified at CDC before being applied in the anthrax emergency has a MDC of 0.06 μg/mL, an RDL of 0.09 μg/mL, and a reactivity threshold of 3.0 μg/mL anti-PA IgG. The reactivity threshold was adopted as the lower limit of quantification. The DSN of the assay is 97.6%, and the DSP is 94.2%. The CI-ELISA enhanced DSP to 100%. These results represent substantial improvements over the published sensitivities for anti-PA ELISAs of 72% (17) and 91.7% (18). Although Harrison et al. (18) reported a specificity of 100%, this was on a sample size of 18 controls, compared with the sample size reported here of 515 control sera (277 non-anthrax-related sera plus 238 NHANES controls). An additional important outcome of this study is the provision of a standard reference serum that can be used in a variety of serologic assays for the detection and quantification of anti-PA antibodies.

When evaluating the importance of a reactive serologic result, the prevalence of disease in the group of interest should first be considered. The assays reported in this study were primarily applied as a part of a panel of laboratory tests for the confirmation of clinical human anthrax in patients in whom the disease prevalence is expected to be high (30). The assays were also applied to serologic surveys of patients who may have been exposed to spores of *B. anthracis*, a group in which the disease prevalence may be expected to be low (13,14). For

assays in which the specificity and sensitivity have been determined to be high (>90%), a reactive serum in a low-prevalence group has a much greater probability of being a false positive than it does in a high-prevalence group (31). Conversely, in the high-prevalence group nonreactive sera may not be indicative of the absence of disease. In practice, the 2.2% frequency of potential false positives reported here rarely presented a problem, and seroconversion was detectable by a ≥4-fold rise in anti-PA IgG concentration above the assay reactivity threshold or the acute-phase serum in all but three patients (data not shown), where paired samples with an appropriate time interval between them were available (0–7 days after symptoms for acute-phase sera and 14–28 days after symptoms for convalescent-phase sera).

The necessity for a rapid public health response meant that optimizing the number and timing of the patient serum collection was not always feasible or practicable. As a result, there was a high frequency of single (i.e., unpaired) sera from cases under investigation. To provide adequate analysis of these single sera and also for paired sera that were reactive but did not demonstrate changing levels of reactivity with time, a competitive rPA inhibition ELISA (CI-ELISA) was developed based on the qualified anti-PA ELISA. The objective of the CI-ELISA was to increase the DSP of the ELISA by reducing the incidence of false positives. The CI-ELISA effectively demonstrated the specificity of the ELISA format by using sera from AVA vaccinees and clinically confirmed anthrax cases (8).

Although very little published information supports the suggestion that antibiotic therapy can suppress the humoral immune response (32), anthrax infection studies in nonhuman primates have shown that early antibiotic treatment after a known challenge with *B. anthracis* spores abrogates an anti-PA antibody response (33). A plausible explanation for this is that early intervention in the infection process minimizes antigen presentation to the immune system. The implication, particularly for cutaneous anthrax in the context of a response to a bioterrorist attack, when antibiotic intervention is likely to be rapid and aggressive (2), is that serologic tests should not be used as the sole confirmatory tests for anthrax.

Conclusion

In this bioterrorism-related anthrax outbreak, the rapid adaptation and laboratory qualification of a quantitative serologic assay for IgG antibodies to the PA component of anthrax toxin contributed to the emergency public health response. The qualified ELISA is accurate, sensitive, specific, reproducible, and quantitative, providing fractional concentrations of anti-PA IgG antibodies. This assay, together with the supplemental CI-ELISA, proved to be an invaluable tool for assisting in early diagnosis of cutaneous and inhalational anthrax cases.

Timing of the sample and specimen quality are critical elements in successful confirmation of anthrax, particularly cutaneous anthrax, where antibiotic therapy has been implemented and the onset of antibody production may be later and of lower magnitude than for inhalational anthrax. To provide an accurate clinical picture on which to base diagnosis and thus treatment, serologic testing is most appropriately used as one of a series of laboratory tests, together with a known exposure or clinical presentation consistent with anthrax.

Ongoing studies on the ability of reactive serum from clinical cases to neutralize anthrax toxin in vitro in a macrophage cytotoxicity assay may help to better describe the complex picture of immune responses to anthrax and determine whether the detection of a serologic response to anthrax toxin PA in humans infected during the bioterrorist attack of fall 2001 indicates protection against further exposure to this disease.

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References

1. Centers for Disease Control and Prevention. Human anthrax associated with an epizootic among livestock—North Dakota, 2000. MMWR Morb Mortal Wkly Rep 2001;50:677–80.
2. Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Anthrax as a biological weapon. JAMA 2002;287:2236–52.
3. Centers for Disease Control and Prevention. Update: cutaneous anthrax in a laboratory worker—Texas, 2002. MMWR Morb Mortal Wkly Rep 2002;51:482.

4. Leppla SH. Purification and characterization of adenylyl cyclase from *Bacillus anthracis*. In: Johnson A, Corbin JD. Methods in enzymology. Vol 195. San Diego: Academic Press;1991. p. 153–68.
5. Reimer CB, Smith SJ, Wells TW, Nakamura RM, Keitges PW, Ritchie RF, et al. Collaborative calibration of the U.S. National and the College of American Pathologists reference preparation for specific serum proteins. Am J Clin Pathol 1982;77:12–9.
6. Madore DV, Johnson CL, Phipps DC, Pennridge Pediatric associates, Myers MG, Eby R, et al. Safety and immunogenicity of *Haemophilus influenzae* type b oligosaccharide-CRM $_{197}$ conjugate vaccine in infants aged 15 to 23 months. Pediatrics 1990;86:527–34.
7. Plikaytis BD, Holder PF, Carlone GM. Program ELISA for Windows user's manual, Version 1.00. Atlanta: Centers for Disease Control and Prevention; 1996.
8. Romero-Steiner S, Elie CM, Holder PF, Semenova V, Helsel L, Thacker L, et al. Specificity of a standardized *Bacillus anthracis* ELISA for measurement of anti-protective antigen IgG antibodies in human sera from individuals diagnosed with cutaneous anthrax. Abstracts of the 4th International Conference on Emerging Infectious Diseases, April 27–30, 2002, Atlanta GA. ICEIDL-667.
9. O'Connell MA, Belanger BA, Haaland PD. Calibration and assay development using the four-parameter logistic model. Chemometrics and Intelligent Laboratory Systems 1993;20:97–114.
10. Plikaytis BD, Turner SH, Gheesling LL, Carlone GM. Comparisons of standard curve fitting methods to quantitate *Neisseria meningitidis* Group A polysaccharide antibody levels by enzyme-linked immunosorbent assay. J Clin Microbiol 1991;29:1439–46.
11. Jacobson RH. Validation of serological assays for diagnosis of infectious diseases. Rev Sci Tech 1998;17:469–526.
12. Plikaytis BD, Holder PF, Pais LB, Maslanka SE, Gheesling LL, Carlone GM. Determination of parallelism and nonparallelism in bioassay dilution curves. J Clin Microbiol 1994;32:2441–7.
13. Hsu VP, Lukacs SL, Handzel T, Hayslett J, Harper S, Hales T, et al. Opening a Bacillus anthracis-containing envelope, Capitol Hill, Washington, D.C.: the public health response. Emerg Infect Dis 2002;8;1039-43.
14. Dewan PK, Fry AM, Laserson K, Tierney BC, Quinn CP, Hayslett JA, et al. Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. Emerg Infect Dis 2002;8;1066-72.
15. Shlyakhov E, Rubinstein E. Evaluation of the Anthraxin skin test for diagnosis of acute and past human anthrax. Eur J Clin Microbiol Infect Dis 1996;15:242–5.
16. Turnbull PCB, Doganay M, Lindeque PM, Aygen B, McLaughlin J. Serology and anthrax in humans, livestock and Etosha National Park wildlife. Epidemiol Infect 1992;108:299–313.
17. Sirisanthana T, Nelson KE, Ezzell JW, Abshire TG. Serological studies of patients with cutaneous and oropharyngeal anthrax from northern Thailand. Am J Trop Med Hyg 1988;39:575–81.
18. Harrison LH, Ezzell JW, Veterinary Laboratory Investigation Center, Abshire TG, Kidd S, Kaufman A. Evaluation of serologic tests for diagnosis of anthrax after an outbreak of cutaneous anthrax in Paraguay. J Infect Dis 1989;160:706–10.
19. Shlyakhov EN, Shvarts SA, Gruz EV. Diagnosis of anthrax by means of Anthraxin. J Hyg Epidemiol Microbiol Immunol 1973;17:279–84.
20. Pfisterer RM. Retrospective verification of the diagnosis of anthrax by means of the intracutaneous skin test with the Russian allergen "Anthraxin" in a recent epidemic in Switzerland. Salisbury Medical Bulletin (Special Supplement) 1990;68:80.
21. Kleine-Albers C, Hönicke E, Böhm R, Pfisterer RM. The detection of antibodies against the protective antigen of *Bacillus anthracis* in infected persons by enzyme-immunoassay (EIA). Salisbury Medical Bulletin (Special Supplement) 1990;68:75–6.
22. Darlow HM, Pride NB. Serological diagnosis of anthrax. Lancet 1969;2:430.
23. Buchanan TM, Feeley JC, Hayes PS, Brachman PS. Anthraxin direct microhemagglutination test. J Immunol 1971;107:1631–6.
24. Strange RE, Thorne CB. Further studies on the protective antigen of *Bacillus anthracis* produced in vitro. J Bacteriol 1958;76:192–202.
25. Johnson-Winegar A. Comparison of enzyme-linked immunosorbent and indirect hemagglutination assays for determining anthrax antibodies. J Clin Microbiol 1984;20:357–61.
26. Iacono-Connors LC, Novak J, Rossi C, Mangiafico J, Ksiazek T. Enzyme-linked immunosorbent assay using a recombinant baculovirus-expresses *Bacillus anthracis* protective antigen (PA): measurement of human anti-PA antibodies. Clin Diagn Lab Immunol 1994;1:78–82.
27. Turnbull PCB, Leppla SH, Broster MG, Quinn CP, Melling J. Antibodies to anthrax toxin in humans and guinea pigs and their relevance to protective immunity. Med Microbiol Immunol 1988;177:293–303.
28. Pittman PR, Kim-Ahn G, Pifat DY, Coonan K, Gibbs P, Little S, et al. Anthrax vaccine: immunogenicity and safety of a dose-reduction, route-change comparison study in humans. Vaccine 2002;20:1412–20.
29. Turnbull PCB, Broster M, Carman AJ, Manchee RJ, Melling J. Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs and their relevance to protective immunity. Infect Immun 1986;52:356–63.
30. Jernigan J, Stephens D, Ashford D, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
31. Jacobson RH. How well do serodiagnostic tests predict the infection or disease status of cats. J Am Vet Med Assoc 1991;199:1343–7.
32. Woo PCY, Tsoi H-W, Won L-P, Leung HC, Yuen K-Y. Antibiotics modulate vaccine-induced humoral immune response. Clin Diagn Lab Immunol 1999;6:832–7.
33. Friedlander AM, Welkos SL, Pitt ML, Ezzell JW, Worsham PL, Rose KJ, et al. Post exposure prophylaxis against experimental inhalational anthrax. J Infect Dis 1993;167:1239–42.

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Molecular Subtyping of *Bacillus anthracis* and the 2001 Bioterrorism-Associated Anthrax Outbreak, United States

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Molecular subtyping of *Bacillus anthracis* played an important role in differentiating and identifying strains during the 2001 bioterrorism-associated outbreak. Because *B. anthracis* has a low level of genetic variability, only a few subtyping methods, with varying reliability, exist. We initially used multiple-locus variable-number tandem repeat analysis (MLVA) to subtype 135 *B. anthracis* isolates associated with the outbreak. All isolates were determined to be of genotype 62, the same as the Ames strain used in laboratories. We sequenced the protective antigen gene (*pagA*) from 42 representative outbreak isolates and determined they all had a *pagA* sequence indistinguishable from the Ames strain (PA genotype I). MLVA and *pagA* sequencing were also used on DNA from clinical specimens, making subtyping *B. anthracis* possible without an isolate. Use of high-resolution molecular subtyping determined that all outbreak isolates were indistinguishable by the methods used and probably originated from a single source. In addition, subtyping rapidly identified laboratory contaminants and nonoutbreak–related isolates.

The recent bioterrorism-associated anthrax outbreak demonstrated the need for rapid molecular subtyping of *Bacillus anthracis* isolates. Numerous methods, including multiple-locus enzyme electrophoresis (MEE) and multiple-locus sequence typing (MLST), have shown the lack of genetic diversity of *B. anthracis* (1–4, unpub. data). Despite this low diversity, methods have been developed that can detect differences between *B. anthracis* isolates. Amplified fragment length polymorphism (AFLP) analysis has been used to detect differences between *B. anthracis* isolates and to examine phylogenetic relationships between *B. anthracis* and its close relatives, *B. cereus* and *B. thuringiensis* (4,5). Keim et al. (6) reported on multiple-locus variable-number tandem repeat analysis (MLVA) for subtyping *B. anthracis*, which unlike AFLP is designed to subtype *B. anthracis* specifically and cannot be used to address phylogenetic relationships between *Bacillus* species. MLVA determines the copy number of variable-number tandem repeats (VNTR) at eight genetic loci (six chromosomal and one on each of the two plasmids). Recently, MLVA has been used to differentiate 426 *B. anthracis* isolates into 89 distinct genotypes and to study the ecology of anthrax (6,7). MLVA is relatively simple, has excellent reproducibility, can subtype multiple strains on a single gel, and gives results in <8 hours.

Protective antigen (PA) is one of the three anthrax toxin proteins and is key to developing immunity to anthrax. Sequencing the gene that encodes PA (*pagA*) has been used to subtype 26 diverse *B. anthracis* isolates into six PA genotypes (8). Although sequencing of *pagA* results in limited numbers of subtypes, it does have the added benefit of determining if the *pagA* gene has been altered or engineered.

During the 2001 bioterrorism-associated anthrax outbreak, we used MLVA to subtype isolates from patients, the environment, and powders. Subtyping of *B. anthracis* allowed anthrax cases to be linked to environmental specimens and powders and provided information about potential sources. Sequencing of *pagA* was also performed on a subset of these *B. anthracis* isolates, and we confirmed that the *pagA* sequence was not altered. In addition, we used these methods on DNA extracted from select clinical specimens to detect and subtype *B. anthracis* directly from clinical specimens. During the outbreak, laboratories throughout the United States and around the world received an increased number of specimens to be tested for *B. anthracis*. With such large numbers, occasional contamination or detection of non-outbreak strains was inevitable, and molecular subtyping was used to clarify these situations on several occasions. Overall, the recent anthrax outbreak has dramatically illustrated the importance of rapid molecular subtyping during a bioterrorism event.

Materials and Methods

During the 2001 anthrax outbreak investigation, 135 *B. anthracis* isolates were subtyped. The identity of all strains was confirmed with standard microbiologic procedures and the Laboratory Response Network (LRN) testing algorithm (9,10). Isolates were obtained from patients with laboratory-

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confirmed anthrax (n=10), powders (n=4), and environmental specimens (n=121). For comparison purposes, five *B. anthracis* isolates originating from New England in the 1960s and 1970s, the Ames strain, and the Pasteur strain were included.

DNA extractions of 28 clinical specimens from six patients with confirmed inhalational anthrax were used for molecular subtyping. These specimens included blood, pleural fluid, blood cultures, serum, cerebrospinal fluid (CSF), lung tissue, and lymph node tissue.

DNA from all strains was prepared with a heat lysis method. Isolates were streaked onto trypticase soy agar containing 5% sheep blood (Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated overnight at 37°C. A single colony was transferred and dispersed into 0.22-µm centrifugal filter units (Millipore, Bedford, MA) containing 200 µL 10 mM Tris-HCl (pH 8.0). The suspension was heated at 95°C for 20 min and cooled to room temperature. The filter units were then centrifuged in a microfuge at 6,000 x *g* for 2 min and the filter discarded. The resulting lysate was stored at –20°C until use. DNA from clinical specimens was extracted with a Qiagen DNA Mini Kit per manufacturer's instructions (Qiagen Inc., Valencia, CA).

MLVA typing was done as described by Keim et al. (6). Briefly, the eight loci were amplified in four reactions: reaction 1 ($vrrB_1$, CG3, and *vrrA*), reaction 2 ($vrrB_2$, pXO1-aat, and pXO2-at), reaction 3 ($vrrC_1$), and reaction 4 ($vrrC_2$). In some instances CG3 was removed from reaction 1 and amplified as a 5th reaction because of weak amplification. Each amplicon was labeled with one of three different dyes. Products were separated by polyacrylamide gel electrophoresis under denaturing conditions on an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA), and allele sizes were determined using ABI Genescan software (Applied Biosystems).

The amplification and sequencing of *pagA* were performed on 42 *B. anthracis* isolates and 22 clinical specimens as described by Price et al. (8), with the following modifications. Initially, synthetic oligonucleotide polymerase chain reaction (PCR) primers PA-1F and PA-1R and PA-2F and PA-2R (Table 1) were used to amplify two overlapping fragments (1,119 bp and 1,449 bp, respectively) together totaling 2,531 bp and containing the *pagA* open reading frame (ORF) (8). Because of inconsistent amplification with PA-2F and PA-2R and to generate a single template for sequencing, PCR amplification was performed using primers 1566F and 4205R. In some instances, possibly from the method of DNA purification, 1566F and 4205R did not amplify sufficiently and thus nested PCR was performed using 1575F and 4191R. The primers used in this study were a combination of both published primers (8) and primers designed from the published DNA sequence of the virulence plasmid pXO1 (GenBank accession no. AF065404) (Table 1). The *pagA* sequencing template was amplified by PCR using the Expand High Fidelity PCR system (Roche, Mannheim, Germany). Fifty-microliter PCR mixtures contained 10 mM Tris-HCL (pH 8.3), 50 mM KCl, 4.0 mM $MgCl_2$, 0.4 mM of each forward and reverse primer, 100 µM of each deoxynucleotide, 2.0 U of *Taq* DNA polymerase (Roche), and 2 µL of bacterial lysate. Reactions were heated at 94°C for 5 min and then cycled 35 times at 94°C for 30 s, 51°C for 30 s, and 72°C for 1.5 min, with a final extension of 72°C for 5 min. PCR amplicons were purified using QIAquick PCR purification kit (Qiagen, Inc.) and the resulting purified amplicons were used in the subsequent sequencing reactions.

Table 1. Primers used for amplification and sequencing of the *pagA* gene

Primer	Sequence (5′ – 3′)
PA1575F[a]	CGA ACT GAT ACA CGT ATT TTA G
PA4191R[a]	AGG ATT ATG ATG ATT TAG ATT ACT
PA1566F[a]	TTT ATC CGA ACT GAT ACA CGT ATT
PA4205R[a]	ACA AAC AAT CTC AAA GGA TTA TGA
PA-1F[a]	ATA TTT ATA AAA GTT CTG TTT AAA AAG CC
PA-1R[a]	TAA ATC CTG CAG ATA CAC TCC CAC
PA-2F[a]	ATA AGT AAA AAT ACT TCT ACA AGT AGG ACA C
PA-2R[a]	GAT TTA GAT TAC TGT TTA AAA CAT ACT CTC C
PA-3	TCA TGT AAC AAT GTG GGT AGA TGA C
PA-4	CTC TAT GAG CCT CCT TAA CTA CTG AC
PA-5F	ATC CTA GTG ATC CAT TAG AAA CGA C
PA-5R	CTT CTC TAT GAG CCT CCT TAA CTA CTG
$PA\text{-}5F_{nest}$	AGT GAT CCA TTA GAA ACG AC
$PA\text{-}5R_{nest}$	TAA CTA CTG ACT CAT CCG C
PA-U2121	TAC ATT TGC TAC TTC CGC TGA TAA
PA-L3892	TGT TTT TCC ATC TTG CCG TAA
2121R	TTA TCA GCG GAA GTA GCA AAT GTA
3892F	TTA CGG CAA GAT GGA AAA ACA
2557R	AGC CGT GCT CCA TTT TTC AGG
3318R	TGC GGT AAC ACT BTCA CTC CAG
2560F	GAA AAA TGG AGC ACG GCT TCT
2924F	CTT GGG CTG AAA CAA TGG GTT

[a]Primers used for amplification of the *pagA* gene. All other primers were used for sequencing.

Sequencing was performed on an Applied Biosystems 3100 genetic analyzer (Applied Biosystems) using BigDye terminator cycle sequencing ready reaction mix according to manufacturer's instructions (Applied Biosystems). All sequence data were analyzed with the Lasergene 99 (DNASTAR, Madison, WI) software, which comprises several different programs: DNA sequences were assembled using the SeqMan program, and MegAlign was used to do sequence comparisons.

Results

By MLVA, all 135 outbreak-related *B. anthracis* isolates had the following loci sizes: *vrrA* = 313, $vrrB_1$ = 229, $vrrB_2$ = 153, $vrrC_1$ = 583, $vrrC_2$ = 532, CG3 = 158, pXO1 = 123, and

pXO2 = 141, resulting in genotype 62, as described by Keim et al. (6). In addition, the entire 2,294-bp *pagA* gene was sequenced from a subset of 42 isolates: including ten patient isolates, all four powder isolates, and 28 select environmental isolates. All isolates had an indistinguishable sequence, PA genotype I (Table 2).

Before *B. anthracis* was detected in the mail, we subtyped several isolates from cutaneous anthrax cases that occurred in the 1960s and 1970s in the eastern United States to determine if any were similar to the outbreak strain. Two isolates from Rhode Island were MLVA genotype 71, one New Hampshire isolate was genotype 78, while an additional New Hampshire isolate and a Massachusetts isolate each had unique combinations of alleles resulting in new genotypes**.** The *pagA* sequence of all five New England isolates was identified as PA genotype VI (Table 2)**.**

State A reported isolating *B. anthracis* (2002017388) from an envelope. This state was not in the vicinity of the 2001 outbreak. By MLVA, the isolate was shown to have been cured of pXO1 and had the same genotype as the Pasteur strain, used in laboratories as a control strain for various tests (Table 2).

Country B sent an isolate (2002007581) that was reportedly isolated from a letter to a private physician. MLVA identified the strain as genotype 45, which clearly distinguished it from the strain associated with the ongoing outbreak in the United States. In addition, four other isolates from the same facility were assayed by MLVA (2002007648–51), resulting in the identification of two Sterne strains, one Pasteur strain, and one additional strain of genotype 45 (Table 2).

MLVA and *pagA* sequencing were performed on clinical specimens collected from seven patients with laboratory-confirmed inhalational anthrax during the 2001 bioterrorism-associated anthrax outbreak. These methods have an unproven utility on clinical specimens, and further testing will be necessary for full evaluation. A total of 28 clinical specimens were analyzed by using MLVA, including: blood, CSF, pleural fluid, serum, lung tissue, and lymph node tissue (Table 3). All eight loci were detected in three specimens (two pleural fluids and one lymph node) from patient 10. Of the eight loci examined, v*rrA* was detected in all nine specimens in which any of the MLVA loci were detected and on two occasions was the only locus detected. The *pagA* gene was successfully amplified and sequenced from 5 of 22 specimens analyzed (Table 3).

Discussion

During the 2001 anthrax investigation, molecular subtyping of *B. anthracis* by MLVA and *pagA* sequencing was important in linking cases to each other and to contaminated sites and in distinguishing isolates that were not related to this event. We used two methods for the molecular subtyping of *B. anthracis*: *pagA* sequencing and MLVA. All outbreak-associated isolates were identified as MLVA genotype 62 and PA genotype I. To date, MLVA genotype 62 has only been associated with a few isolates from herbivores in Texas and has not been identified in any *B. anthracis* strains originating in eastern United States or anywhere else in the world. None of the New England isolates analyzed in this study were MLVA genotype 62 or PA genotype I. All five were of PA genotype VI, while MLVA identified two isolates as genotype 71, one as genotype 78, and two as new genotypes. Genotype 62 is also the genotype of the Ames strain commonly used in research laboratories worldwide and frequently used in animal

Table 2. MLVA and *pagA* genotyping of *Bacillus anthracis* isolates[a,b]

B. anthracis strain	No. strains	vrrA	$vrrB_1$	$vrrB_2$	$vrrC_1$	$vrrC_2$	CG3	pXO1	pXO2	MLVA type	PA genotype
Outbreak-associated	135	313	229	153	583	532	158	123	141	62	I
Ames	1	313	229	153	583	532	158	123	141	62	I
NH (2000032764)	1	301	229	153	538	604	158	132	139	78	VI
NH (2000032760)	1	313	229	153	538	604	158	123	139	New[e]	VI
RI (2000032763)	1	313	229	162	538	604	158	132	139	71	VI
RI (2000032761)	1	313	229	162	538	604	158	132	139	71	VI
MA (2000032762)	1	313	229	153	538	604	158	132	143	New[e]	VI
State A (2002017388)	1	313	229	162	613	604	153	–	137	Pasteur[c]	NA[d]
Pasteur	1	313	229	162	613	604	153	–	137	Pasteur[c]	NA[d]
Country B (2002007581)	1	313	229	162	613	532	158	129	141	45	I
Country B (2002007648)	1	313	229	162	613	532	158	129	141	45	I
Country B (2002007649)	1	313	229	162	613	604	153	–	137	Pasteur[c]	NA[d]
Country B (2002007650)	1	313	229	162	583	532	158	129	_	Sterne[c]	I
Country B (2002007651)	1	313	229	162	583	532	158	129	_	Sterne[c]	I

[a]MLVA, multiple-locus variable-number tandem repeat analysis; *pagA*, protective antigen gene; PA, protective antigen; –, loci not detected; NA, not applicable.
[b]Allele size for each VNTR locus is shown in addition to the MLVA and PA genotypes.
[c]No MLVA genotype assigned due to the lack of one of the virulence plasmids (pXO1 or pXO2).
[d]*pagA* not present in pXO1-cured strains and thus could not be assigned a PA genotype.
[e]New combination of alleles resulting in a new genotype. Genotype no. to be assigned at a later date.

Table 3. Molecular subtyping by MLVA and *pagA* sequencing performed on 28 clinical specimens from seven patients with inhalational anthrax[a,b]

Patient no.[b]	Specimen type	Interval after anti-microbial therapy (days)[d]	MLVA loci detected	*pagA*	*Bacillus anthracis* LRN PCR[e]
1	Pleural fluid[c]	4	All negative	Negative	Positive
	Pleural fluid[c]	4	All negative	Negative	Positive
	Blood[c]	4	All negative	Negative	Negative
	Lung[c]	4	All negative	ND	ND
	Lung[c]	4	*vrrA*	ND	Negative
	Heart blood[c]	4	*vrrA*, $vrrB_1$	Negative	Negative
	Pericardial blood[c]	4	All negative	Negative	Positive
2	Thoracentesis fluid	4	v*rrA*	Negative	Positive
	Serum	10	All negative	Negative	Positive
	Respiratory wash	4	All negative	Negative	Positive
	Pleural fluid	4	All negative	Negative	Positive
3	Blood culture	0	All negative	Negative	Positive
5	Blood culture	0	All negative	Negative	Positive
	Blood culture	0	All negative	Negative	Positive
6	Blood culture	0	*vrrA,* $vrrB_1$, $vrrB_2$,$vrrC_2$	Negative	Positive
10	Pleural fluid	1	*vrrA*, $vrrB_1$, $vrrB_2$, $vrrC_1$, $vrrC_2$, CG3, pXO1, pXO2	Positive	Positive
	Pleural fluid	1	*vrrA*, $vrrB_1$, $vrrB_2$, $vrrC_1$, $vrrC_2$, CG3, pXO1, pXO2	Positive	Positive
	Blood	1	All negative	Negative	Positive
	CSF[c]	3	All negative	Negative	Positive
	Lung[c]	3	*vrrA*, CG3	Negative	Positive
	Lymph node[c]	3	*vrrA*, $vrrB_1$, $vrrB_2$, $vrrC_1$, $vrrC_2$, CG3, pXO1, pXO2	Positive	Positive
11	Pleural fluid	2	All negative	Positive	Positive
	Blood	2	All negative	Negative	Negative
	Blood culture	-1	All negative	ND	ND
	Blood culture	-1	All negative	ND	Positive
	Blood culture	-1	All negative	ND	ND
	Blood culture	-1	All negative	ND	Positive
	Lymph node[c]	4	*vrrA*, $vrrB_1$, $vrrB_2$, $vrrC_1$, $vrrC_2$, CG3	Positive	Positive

[a]MLVA, multiple-locus variable-number tandem repeat analysis; *pagA*, protective antigen gene; LRN, Laboratory Response Network; PCR, polymerase chain reaction.
[b]Patients 1–10 described in Jernigan et al. (19) and patient 11 in Barakat et al (20)
[c]Specimens collected postmortem.
[d]Number of days the specimen was collected following or before the initiation of antimicrobial therapy. Specimens collected the same day as the initiation of therapy were designated as day 0 but were collected before antibiotic therapy.
[e]Results using the Laboratory Response Network PCR assay for detection of *B. anthracis* during the outbreak (18).

challenge studies (11–16). The sequence of *pagA* from the outbreak strain, PA genotype I, was also identical to that of the Ames strain; thus, the outbreak *B. anthracis* strain is indistinguishable from the Ames strain based on the examination of the eight MLVA loci and the *pagA* sequence. Recently, comparative genome sequencing detected only four differences between the chromosomes of the outbreak strain (Florida isolate) and Ames (Porton) isolate (17).

Molecular subtyping of isolates immediately upon their arrival to the laboratory allowed for instant confirmation that the cases were caused by the same strain and thus for linking cases to environmental contamination and to the powder-containing envelopes. The speed of the MLVA allowed for genotype identification within 8 hours of receiving the isolates. In addition to linking the cases, molecular subtyping was invaluable in determining if *B. anthracis,* isolated from around the

world during the same time period, were potentially related to the ongoing outbreak in the United States. The level of discrimination provided by MLVA, allowed for non-outbreak isolates to be rapidly and easily distinguished.

While both MLVA and *pagA* sequencing are primarily used for molecular subtyping of isolates, we were also able to amplify the eight MLVA loci and *pagA* directly from a limited number of available clinical specimens. Although this event was not a prospective case-control study, amplification was most successful from pleural fluid and lymph node specimens. Similar results were demonstrated with a *B. anthracis*-specific real-time PCR assay (18). Amplification of the MLVA loci and *pagA* was not very successful from blood cultures even when taken before antibiotic therapy. The lack of success with blood cultures was not because of a complete inhibition of PCR since the *B. anthracis* LRN PCR assay was positive on these specimens. Of the MLVA loci, *vrrA* was the most readily amplified (9 of 28). This is likely the result of a lower limit of detection for *vrrA* compared to the other loci; however, limits of detection for each of the loci have not been evaluated.

For a single patient (patient 10), we were able to amplify all eight MLVA loci and determine the genotype of the *B. anthracis* strain without having the isolate itself. In this instance, MLVA was used directly on DNA extracted from pleural fluid and genotype 62 was identified. *B. anthracis* was not successfully cultured from that same pleural fluid sample. The *pagA* gene was amplified and sequenced from the same DNA specimen and identified as PA genotype I. MLVA and *pagA* amplification were attempted on DNA extracted from blood drawn from this patient the same day as the pleural fluid but failed to detect any of the loci, suggesting more efficient clearance of the bacilli from the blood or less sensitivity of these molecular approaches on blood compared to pleural fluid. Again, the negative result on blood was not because of complete inhibition of PCR since the *B. anthracis* LRN PCR assay on this sample was positive. At a later date, when the Centers for Disease Control and Prevention received the isolate originally cultured from this patient at the local medical facility where the patient was treated, the isolate was confirmed to be genotype 62. Despite the fact that *B. anthracis* was not successfully cultured from any of these clinical specimens taken after the initiation of antimicrobial therapy, we were able to amplify the MLVA loci and *pagA* from some of these specimens.

The entire chromosomal sequence of the *B. anthracis* Ames strain (available from: URL: www.tigr.org) is now available and has been compared to the chromosomal sequence of the outbreak (Florida) isolate (17). While sequencing and comparing *B. anthracis* genomes are not likely to be useful for rapidly identifying isolates during an outbreak investigation, the data generated from such comparisons may identify new loci, which could be targets for methods such as MLVA and can be done rapidly on large numbers of isolates from patients, the environment, and on DNA from clinical specimens.

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Dr. Hoffmaster is a microbiologist in the Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention. His interests include research on methods for rapid detection and molecular subtyping of *Brucella* spp., *Burkholderia* spp., and *Bacillus anthracis*.

References

1. Harrell LJ, Andersen GL, Wilson KH. Genetic variability of *Bacillus anthracis* and related species. J Clin Microbiol 1995;33:1847–50.
2. Henderson ID, Yu D, Turnbull PC. Differentiation of *Bacillus anthracis* and other '*Bacillus cereus* group' bacteria using IS231-derived sequences. FEMS Microbiol Lett 1995;128:113–8.
3. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, et al. *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*—one species on the basis of genetic evidence. Appl Environ Microbiol 2000;66:2627–30.
4. Keim P, Kalif A, Schupp J, Hill K, Travis SE, Richmond K, et al. Molecular evolution and diversity in *Bacillus anthracis* as detected by amplified fragment length polymorphism markers. J Bacteriol 1997;179:818–24.
5. Ticknor LO, Kolsto AB, Hill KK, Keim P, Laker MT, Tonks M, et al. Fluorescent amplified fragment length polymorphism analysis of Norwegian *Bacillus cereus* and *Bacillus thuringiensis* soil isolates. Appl Environ Microbiol 2001;67:4863–73.
6. Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. J Bacteriol 2000;182:2928–36.
7. Smith KL, DeVos V, Bryden H, Price LB, Hugh-Jones ME, Keim P. *Bacillus anthracis* diversity in Kruger National Park. J Clin Microbiol 2000;38:3780–4.
8. Price LB, Hugh-Jones M, Jackson PJ, Keim P. Genetic diversity in the protective antigen gene of *Bacillus anthracis*. J Bacteriol 1999;181:2358–62.
9. Khan AS, Morse S, Lillibridge SR. Public-health preparedness for biological terrorism in the USA. Lancet 2000;356:1179–82.
10. Logan NA, Turnbull PC. *Bacillus* and recently derived genera. In: Murray P, editor. Manual of clinical microbiology. Washington: ASM Press; 1991. p. 357–69.
11. McBride BW, Mogg A, Telfer JL, Lever MS, Miller J, Turnbull PC, et al. Protective efficacy of a recombinant protective antigen against *Bacillus anthracis* challenge and assessment of immunological markers. Vaccine 1998;16:810–7.
12. Barnard JP, Friedlander AM. Vaccination against anthrax with attenuated recombinant strains of *Bacillus anthracis* that produce protective antigen. Infect Immun 1999;67:562–7.
13. Little SF, Ivins BE, Fellows PF, Friedlander AM. Passive protection by polyclonal antibodies against *Bacillus anthracis* infection in guinea pigs. Infect Immun 1997;65:5171–5.
14. Welkos S, Little S, Friedlander A, Fritz D, Fellows P. The role of antibodies to *Bacillus anthracis* and anthrax toxin components in inhibiting the early stages of infection by anthrax spores. Microbiol 2001;147:1677–85.
15. Ivins BE, Pitt ML, Fellows PF, Farchaus JW, Benner GE, Waag DM, et al. Comparative efficacy of experimental anthrax vaccine candidates against inhalation anthrax in Rhesus macaques. Vaccine 1998;16:1141–8.
16. Ivins B, Fellows P, Pitt L, Estep J, Farchaus J, Friedlander A, et al. Experimental anthrax vaccines: efficacy of adjuvants combined with protective antigen against an aerosol *Bacillus anthracis* spore challenge in guinea pigs. Vaccine 1995;13:1779–84.
17. Read TD, Salzberg SL, Pop M, Shumway M, Umayam L, Jiang L, et al. Comparative genome sequencing for discovery of novel polymorphisms in *Bacillus anthracis.* Science 2002;296:2028–33.

18. Hoffmaster AR, Meyer RF, Bowen M, Marston CK, Weyant RS, Barnett GA, et al. Evaluation and validation of a real-time polymerase chain reaction assay for rapid identification of *Bacillus anthracis*. Emerg Infect Dis 2002;8;1178-82.
19. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
20. Barakat LA, Quentzel HL, Jernigan JA, Kirschke DL, Griffith K, Spear SM, et al. Fatal inhalational anthrax in a 94-year-old Connecticut woman. JAMA 2002;287:863–68.

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Sequencing of 16S rRNA Gene: A Rapid Tool for Identification of *Bacillus anthracis*

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In a bioterrorism event, a tool is needed to rapidly differentiate *Bacillus anthracis* from other closely related spore-forming *Bacillus* species. During the recent outbreak of bioterrorism-associated anthrax, we sequenced the 16S rRNA genes from these species to evaluate the potential of 16S rRNA gene sequencing as a diagnostic tool. We found eight distinct 16S types among all 107 16S rRNA gene sequences that differed from each other at 1 to 8 positions (0.06% to 0.5%). All 86 *B. anthracis* had an identical 16S gene sequence, designated type 6; 16S type 10 was seen in all *B. thuringiensis* strains; six other 16S types were found among the 10 *B. cereus* strains. This report describes the first demonstration of an exclusive association of a distinct 16S sequence with *B. anthracis*. Consequently, we were able to rapidly identify suspected isolates and to detect the *B. anthracis* 16S rRNA gene directly from culture-negative clinical specimens from seven patients with laboratory-confirmed anthrax.

The gram-positive, rod-shaped, and spore-forming bacterium *Bacillus anthracis* is the cause of the acute and often lethal disease anthrax. Phenotypic characteristics commonly used to differentiate *B. anthracis* from closely related *B. cereus* and *B. thuringiensis*, such as susceptibility to ß-lactam antibiotics, lack of motility, lack of hemolysis on sheep blood agar (SBA) plate, and susceptibility to γ-phage lysis, may vary among different *Bacillus* species strains, hampering their identification and differentiation. Phenotypically and genotypically *B. thuringiensis* can be differentiated from *B. cereus* by the presence of the CRY crystal protein and plasmid-encoded *cry* genes (1), but if this plasmid were lost, *B. thuringiensis* could no longer be distinguished from *B. cereus* (1). The sequence of the 16S rRNA gene has been widely used as a molecular clock to estimate relationships among bacteria (phylogeny), but more recently it has also become important as a means to identify an unknown bacterium to the genus or species level. The 16S rRNA gene sequences of *B. anthracis*, *B. cereus*, and *B. thuringiensis* have high levels of sequence similarity (>99%) that support their close relationships shown by DNA hybridization (2–7). A limited number of 16S rRNA sequences of *B. anthracis* (7 sequences), *B. cereus* (34 sequences), and *B. thuringiensis* (16 sequences) have been available at GenBank. Although those sequences are of different lengths and qualities, in complementary regions they differ from each other by no more than a few nucleotides. Therefore, this minimal level of diversity seen in the 16S rRNA of *B. anthracis*, *B. cereus*, and *B. thuringiensis* was thought to be an obstacle for using 16S rRNA gene sequencing to identify and differentiate these three species. The bioterrorism events of October 2001 prompted us to evaluate several new molecular approaches to rapidly identify *B. anthracis.* We determined the entire 16S rRNA sequences in a large number of representative strains of *B. anthracis, B. cereus,* and *B. thuringiensis* to evaluate the potential of 16S rRNA sequencing not only to rapidly identify *B. anthracis* in culture, but also to detect *B. anthracis* directly in clinical specimens.

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Materials and Methods

Bacterial Strains

A total of 107 strains were included in this study. Of 86 *B. anthracis* isolates analyzed (Table 1), 18 were selected to represent a wide range of temporal (1937–1997), geographic (16 countries), and source diversity (soil, animals, or humans). Fourteen reference and standard strains, such as the Vollum, Ames, Pasteur, New Hampshire, V770, and Sterne strains, were also included. The remaining 54 strains were isolated from October to December 2001 during the bioterrorism-associated anthrax outbreak in the United States. Ten *B. cereus* and 11 *B. thuringiensis* strains were also analyzed by 16S rRNA sequencing. All strains were identified by standard microbiologic procedures and according to the Laboratory Response Network diagnostic criteria (9,10).

Clinical Specimens

We analyzed 198 clinical specimens (76 blood, 30 tissue, 16 pleural fluid, 37 serum, 6 cerebrospinal fluid, and 33 other specimens). Sixty-nine specimens were obtained from patients with laboratory-confirmed anthrax (55 specimens from 11 inhalational cases and 14 from 7 cutaneous cases). DNA was extracted from fluid (200 µL) or small tissue specimens (<5 mm^3) according to manufacturer's instructions with a Qiagen DNA Mini Kit (Qiagen, Valencia, CA). All 198 DNA samples

Table 1. Descriptions and GenBank accession numbers of 107 *Bacillus* species strains analyzed in this study

Species		No.	Identification	GenBank 16S rRna gene accession number	Geographic and/or temporal origin[a]	16S rRNA type	MLVA genotype[b]
B. anthracis	Diversity collection	18	2000031650	AY138379	Human, Turkey, 1991	6	23
			2000031651	AY138372	Bovine, France, 1997	6	80
			2000031652	AY138374	Human, US, 1952	6	68
			2000031653	AY138373	Wool, Pakistan, 1976	6	69
			2000031655	AY138376	Cow, China	6	57
			2000031656	AY138375	Ames	6	62
			2000031657	AY138382	Bovine	6	10
			2000031659	AY138377	Human, Turkey, 1984	6	28
			2000031660	AY138378	Bovine, US, 1937	6	25
			2000031661	AY138369	Human, South Korea, 1994	6	34
			2000031662	AY139368	Zebra, Namibia	6	35
			2000031663	AY138381	Bovine, Poland	6	15
			2000031664	AY138383	Porcine, German, 1971	6	38
			2000031665	AY138366	Bovine, Argentina	6	45
			2000031666	AY138371	UK	6	77
			2000031667	AY138380	Sheep, Italy, 1994	6	20
			2000031670	AY138367	Human, Turkey, 1985	6	41
			2000031671	AY138370	Bovine, Zambia	6	30
	Standard and reference strains	14	Ames	AY138358	Ames	6	62
			2002007651	AY138355	Sterne, Chile	6	ND
			2002007650	AY138356	Sterne, Chile	6	ND
			2002007649	AY138357	Pasteur, Chile	6	ND
			2000031887B	AY138347	Vaccine	6	ND
			2000031666	AY138352	Vollum	6	77
			2000031368	AY138350	Vollum	6	77
			2000031244	AY138354	Vollum	6	*
			2000031078	AY138351	Vollum M36	6	ND
			2000031076	AY138353	Vollum	6	*
			2000031259	AY138346	Pasteur	6	**
			2000031075	AY138345	Sterne	6	*
			2000031887	AY138348	V770-NP1-R	6	*
			2000031136	AY138349	New Hampshire	6	73
	Outbreak strains	54		AY138291 to AY138344	US Oct/Dec 2001	6	62
B. cereus		10	2000031486	AY138272	Human, US, 1994	12	NA
			2000031491	AY138276	Human, US, 1997	7	NA
			2000031498	AY138274	Human, US, 1979	9	NA
			2000031503	AY138277	Human, US, 1999	7	NA
			2000031513	AY138279	Human, US, 1986	13	NA
			G3317	AY138278	Human, Israel, 1989	7	NA
			G8639	AY138271	Milk, Bolivia, 1993	3	NA
			G9667	AY138273	Human, US, 1995	12	NA
			H1439	AY138270	Human, US, 2000	2	NA
			ATCC 14579	AY138275	1887	9	NA
B. thuringiensis		11	2000031482	AY138290	Human, US, 1989	10	NA
			2000031485	AY138289	Spray, US, 1993	10	NA
			2000031494	AY138288	Human, US, 1985	10	NA
			2000031496	AY138287	Human, US, 1981	10	NA

Table 1 continued. Descriptions and GenBank accession numbers of 107 *Bacillus* species strains analyzed in this study

Species	No.	Identification	GenBank 16s rRna gene accession number	Geographic or temporal origin[a]	16S rRNA type	MLVA genotype
		2000031508	AY138286	Human, US, 1985	10	NA
		2000031509	AY138285	Human, US, 1985	10	NA
		2002007400	AY138283	Powder, US, 2001	10	NA
		2002017401	AY138284	Powder, US, 2001	10	NA
		2000032755	AY138282	Environment, US, 2000	10	NA
		2000032757	AY138280	Environment, US, 2000	10	NA
		2000032756	AY138281	Human, US, 1981	10	NA

[a] Date and source of isolation are provided when available; *, lacking pXO2; **, lacking pXO1.
[b]ND, MLVA (8).
NA, not applicable.

were analyzed for 16S rRNA gene amplification and products sequenced.

Polymerase Chain Reaction (PCR)

A 1,686-bp fragment of DNA, including the 1,554-bp 16S rRNA gene, was amplified from all 107 *Bacillus* species strains by using primers 67F and 1671R (Table 2). For clinical samples, we used the initial DNA amplicon as a template in a nested PCR with a second set of internal primers, 23F and 136R (Table 1). Both sets of primers were designed from the *B. anthracis* genome sequence (http://www.tigr.org). The full-length size of *B. anthracis* 16S rRNA gene (1,554 bp) was determined from an alignment of the 16S rRNA genes from *Escherichia coli*, *Neisseria gonorrhoeae* (GenBank accession nos. J01859 and X07714, respectively), and the 16S rRNA gene regions of the *B. anthracis* genome sequence (http://www.tigr.org). Whole cell suspensions or DNA extracts were used for PCR of isolates or clinical samples, respectively. For whole cell suspensions, a single colony from an SBA plate was resuspended in 200 µL of 10 mM Tris, pH 8.0. The suspension was put in a Millipore 0.22-µm filter unit (Millipore, Bedford, MA), heated at 95°C for 20 min, centrifuged at 8,000 rpm for 2 min, and then used for PCR. Each final PCR reaction (100 µL) contained 5 U of Expand DNA polymerase (Roche, Mannheim, Germany); 2 µL of whole cell suspension or DNA; 10 mM Tris-HCl (pH 8.0); 50 mM KCl; 1.5 mM $MgCl_2$; 200 µM (each) dATP, dCTP, dGTP, and dTTP; and 0.4 µM of each primer. Reactions were first incubated for 5 min at 95ºC. Then 35 cycles were performed as follows: 15 s at 94ºC, 15 s at the annealing temperature of 52ºC, and 1 min 30 s at 72ºC. Reactions were then incubated at 72ºC for another 5 min. The annealing temperature for the nested PCR was 50ºC. PCR products were purified with Qiaquick PCR purification kit (Qiagen).

16S rRNA Sequence Determination

The amplified products of approximately 1,686 bp (1,656 bp for nested PCR) were sequenced by using a modification of 16 primers as described (Table 2) (11). Sequencing was performed by using a Big Dye terminator cycle sequencing kit (Applied BioSystems, Foster City, CA). Sequencing products were purified by using Centri-Sep spin columns (Princeton Separations, Adelphia, NJ) and were resolved on an Applied BioSystems model 3100 automated DNA sequencing system (Applied BioSystems). The length of sequences obtained differed for each primer but were sufficient to provide 5- to 8-fold sequence coverage. An inner fragment of 1,554 bp was

Table 2. Primers used for amplification and sequencing of the 16S rRNA gene of *Bacillus anthracis*, *B. thuringiensis*, and *B. cereus*[a]

Generic primers used for 16S rRNA amplification	
8F	5′AGT TGA TCC TGG CTC AG 3′
1492R	5′ACC TTG TTA CGA CTT3′
Primers for amplification of the 16S rRNA gene	
67F	5′TGA AAA CTG AAC GAA ACA AAC 3′
1671R	5′CTC TCA AAA CTG AAC AAA ACG AAA 3′
Inner primers used for nested PCR on clinical samples	
23F	5′ACA AAC AAC GTG AAA CGT CAA 3′
136R	5′AAA CGA AAC ACG GAA ACT T 3′
Primers used for sequencing of the 16S rRNA gene	
104F	5′GGA CGG GTG AGT AAC ACG TG 3′
104R	5′CAC GTG TTA CTC ACC CGT CC 3′
1230F	5′TAC ACA CGT GCT ACA ATG 3′
1390F	5′GGG CCT TGT ACA CAC CG 3′
1390R	5′CGG TGT GTA CAA GGC CC 3′
8F	5′AGT TGA TCC TGG CTC AG 3′
357F	5′TAC GGG AGG CAG CAG 3′
357R	5′CTG CTG CCT CCC GTA 3′
530F	5′CAG CAG CCG CGG TAA TAC 3′
530R	5′GTA TTA CCG CGG CTG CTG 3′
790F	5′ATT AGA TAC CCT GGT AG 3′
790R	5′CTA CCA GGG TAT CTA AT 3′
981F	5′CCC GCA ACG AGC GCA ACC C 3′
981R	5′GGG TTG CGC TCG TTG CGG G 3′

[a]Primers 67F and 1671R or primers 23F and 136R were also used for 16S sequence on isolates or clinical samples, respectively.

obtained and analyzed by using the GCG (Wisconsin) Package, v. 10.1, (Genetics Computer Group, Madison, WI). A number was assigned for each allele of 16S rRNA gene sequence in order of elucidation; a single base change or a mixed base (more than one nucleotide determined at a single position) was considered a new 16S type. When a novel 16S type, mixed base pairs, or any discrepancies in the alignment were obtained, the 16S rRNA gene amplification and sequencing of the entire gene or parts containing the problematic region were repeated.

GenBank 16S rRNA Gene Sequences and Accession Numbers

Sixty 16S rRNA gene sequences of *B. anthracis*, *B. cereus,* and *B. thuringiensis* were available in GenBank. Thirty-nine of these sequences were incomplete, contained a large number of undetermined nucleotides, or were not associated with a specific strain identification, and therefore were not used in this study. The remaining 21 sequences were identified as eight *B. anthracis* (AF155950 [Ames]), (AF155951 [Delta Ames]), (AF176321 [Sterne]), (AF290552 [Sterne]), (AF290553 [Vollum]), (AF155950 [Ames]), (AF155951 [Delta Ames]), and (AF176321 [Sterne]); eight *B. cereus* (AF155952, AF155958, AF176322, AF290546, AF290547, AF290548, AF290550, and AF290555); three *B. thuringiensis* (AF155954, AF155955, and AF290549); and two *B. mycoides* (AF155956 and AF155957). A total of 114 16S rRNA gene sequences were determined in this study (107 from isolates [GenBank accession nos. in Table 1] and 7 from clinical specimens [GenBank accession nos. AY138359 to AY138365).

Results

16S rRNA Gene Sequence Diversity

The 1,554-bp nucleotide sequences of the entire 16S rRNA gene from all 107 *Bacillus* species strains were aligned and compared. Differences were found at eight single nucleotide positions (positions 1, 2, 3, 4, 6, 9, 12, and 13), and no gaps were present. When 21 *Bacillus* 16S rRNA sequences from GenBank were added to the alignment, five additional positions with differences (positions 5, 7, 8, 10, and 11) were located (Table 3). The 13 positions of differences were distributed throughout the gene (Table 3). In six of these positions (positions 1, 2, 3, 4, 6, and 12), more than one nucleotide was detected (mixed nucleotides) (Table 3). These results indicated that the strain contained multiple rRNA operons with slightly different 16S rRNA gene sequences.

We found eight different 16S types among the 107 16S rRNA genes from our collection of isolates (Table 3). All 86 *B. anthracis* had an identical sequence, 16S type 6, containing a single mixed base, a W(A/T) at position 12, not found in the other two species. 16S type 10 was seen in all 11 *B. thuringiensis* strains, and a single mixed base pair was identified in all strains at position 6. Six other 16S types were found among the 10 *B. cereus* strains. Three additional 16S types were found among the 18 GenBank sequences that we analyzed. 16S types 1, 4, and 5 correlated to *B. mycoides*, *B. thuringiensis*, and *B. cereus,* respectively (Table 3). Five *B. anthracis* sequences from GenBank were identical to the 16S type 6 found in all our 86 *B. anthracis* isolates, and three were identical to the 16S type 7 found in *B. cereus*.

16S rRNA Sequencing Directly in Clinical Specimens

We detected 16S rRNA genes in 7 (3.5%) of 198 clinical samples: all were 16S type 6 characteristic for *B. anthracis*. None of the seven specimens were culture positive (Table 4), although all specimens had been collected from patients with laboratory-confirmed anthrax.

Discussion

The goal of this study was to evaluate the potential of 16S rRNA sequencing to rapidly identify *B. anthracis* in cultures. We found that 16S rRNA genes of *B. anthracis* were highly conserved; only one 16S type (16S type 6) was identified in all 86 strains tested. However, not all *B. anthracis* 16S rRNA genes sequences in GenBank are type 6. Three of the eight *B. anthracis* 16S rRNA sequences are reported as type 7, a type that, in our study, we found exclusively among the *B. cereus* strains. The only difference between type 7 and type 6 is a mixed base pair at position 1146. The strain designations of two of these three 16S type 7 *B. anthracis* strains in GenBank are Ames and Sterne. We did not acquire these particular strains from the submitting laboratory, but the one Ames and two Sterne strains (obtained from different sources) in our collection were consistently type 6. A third Sterne strain 16S rRNA sequence in GenBank is also type 6.

One possible explanation for these different 16S rRNA sequencing results may be the use of different sequencing approaches, such as using cloned DNA versus genomic DNA as template. In sequencing clones, one allele may be missed if only a few clones are sequenced, not representing the total diversity. In this case, the position with the mixed base would not be detected. If both types 6 and 7 exist in *B. anthracis*, the difference may be due to recombination, mutation, or loss of an allele. The type 7 *B. anthracis* sequences in GenBank are unpublished; therefore, we do not know if the genes were cloned and, if so, how many clones were sequenced.

The complete *B. anthracis* genome was posted at http://www.tigr.org/tigr-scripts/ufmg/ReleaseDate.pl on May 7, 2002. The genome has 11 rRNA operons. There are 10 positions in the 16S rRNA gene where the nucleotides are not identical among the 11 rRNA operons, but the DNA sequencing software scores only one of them as a mixed base 100% of the time. This position is 1146, where five 16S rRNA genes contain Ts and six have As in a 54%:46% ratio. In this case, the base-calling software (GCG; Genetics Computer Group) always assigns a W at that position. At position 1137, there are seven Gs and four As, a 64%:36% ratio, but the position is scored as a G, the predominant base. In eight positions, a 9%:91% ratio is present. For example, at position 1047 are one

Table 3. 16S rRNA gene types identified among 125 *Bacillus* spp. strains analyzed in this study (n=107) and available at GenBank (n=18)

			Positions[a]												
16S type	*Bacillus* species	No. of strains	1 (77)	2 (90)	3 (92)	4 (182)	5 (189)	6 (192)	7 (200)	8 (208)	9 (1,015)	10 (1,036)	11 (1,045)	12 (1,146)	13 (1,462)
16S types identified in 107 strains in this study															
2	*cereus*	1	R[b]	Y	W	C[c]	A	T	T	G	A	T	A	A	A
3	*cereus*	1	G	C	A	Y	A	T	T	G	A	T	A	A	A
6	*anthracis*	86	A	T	T	C	A	C	T	G	C	T	A	W	T
7	*cereus*	3	A	T	T	C	A	C	T	G	C	T	A	A	T
9	*cereus*	2	A	T	T	C	A	C	T	G	A	T	A	A	T
10	*thuringiensis*	11	A	T	T	C	A	Y	T	G	A	T	A	A	T
12	*cereus*	2	A	T	T	Y	A	T	T	G	A	T	A	A	T
13	*cereus*	1	A	T	T	C	A	C	T	G	C	T	A	T	T
16S types identified in strains available at GenBank[d]															
1	*mycoides*	2	A	T	T	C	C	C	G	C	C	C	G	A	-[e]
4	*thuringiensis*	3	G	C	A	C	A	T	T	G	A	T	A	A	-
5	*cereus*	8	G	C	A	C	A	C	T	G	A	T	A	A	-
7	*anthracis*	3	A	T	T	C	A	C	T	G	C	T	A	A	T

[a]Numbers refer to the number of positions where mismatches are found. Numbers in parentheses refer to positions in the 16S rRNA gene.
[b]R refers to a purine (A or G) at that position; Y refers to a pyrimidine (C or T) at that position; and W refers to an A or T at that position.
[c]A, C, G, and T refer to the four deoxynucleotides that DNA comprises.
[d]Five additional positions of differences (positions 5, 7, 8, 10, and 11) were found when GenBank sequences were used.
[e]The last position (position 13) on 16S types 1, 4, and 5 is missing because those GenBank sequences are shorter.

T and 10 Cs. In these cases, the nucleotide is called as the predominant base by the base-calling software.

The quality of DNA sequences generated in laboratories has been greatly improved by the introduction of automated sequencing systems and DNA alignment software, but other factors, such as the purity of the DNA template and number of overlapping nucleotide fragments in the alignment, contribute to the reliability of the final sequence. Mixed base pairs are clearly the result of sequence differences between different rRNA operons and not due to any sequencing artifacts. In this study, the length of the fragment sequences varied for each primer, but they were of sufficient length to provide 5- to 8-fold sequence coverage in both directions. This 5–8 sequence overlap simplifies identifying and clarifying positions with double signals, increasing the confidence in our final consensus sequence. The occurrence of mixed base pairs in rRNA sequences is well known and accepted (15–19). The Ribosomal Database Project Web site shows that operon heterogeneity has been documented in several different bacterial species (http://rrndb.cme.msu.edu/rrndb/rrn_table.pdf). In addition, we did not observe mixed base pairs in single-copy genes such as *pagA* and a variety of others. A previous study of a small set of *Bacillus* strains isolated from soil demonstrated the diversity of 16S rRNA genes of both *B. cereus* and *B. thuringiensis* (15). Our results confirm the diversity among *B. cereus* strains, although we did not find diversity among *B. thuringiensis* strains. The lack of diversity in our collection of *B. thuringiensis* strains may be associated with natural selection with human host; 6 of 11 of our *B. thuringiensis* strains were isolated from humans.

Direct Amplification of 16S rRNA from Clinical Samples

Even though *B. anthracis* is present at high levels (up to 10^8/mL) in the blood of patients with anthrax and will readily grow on standard bacteriologic media, as for other bacteria, specimens collected after the administration of antimicrobial therapy may fail to grow *B. anthracis*. Laboratory confirmation for the two patients with inhalational anthrax whose specimens were analyzed (patient #10i [12], and patient #11i [13]) was achieved by isolation and identification of *B. anthracis* from clinical samples at the medical facility where the patients were treated. Generally, for all patients, isolates themselves were forwarded to the appropriate public health laboratory and then to the Centers for Disease Control and Prevention for confirmatory identification and molecular subtyping, but the initial clinical specimens were not sent along with the isolates. With few exceptions, clinical specimens available for analysis from these two patients and from other patients with inhalational anthrax were collected after initiation of antimicrobial therapy, resulting in few culture-positive results. For 3 of the 11 inhalational patients, laboratory confirmation was based on two of three available supportive tests, including PCR targeting two plasmid and one chromosomal target (14), immunohistochemistry or a reactive anti-protective antigen titer (immunoglobulin G ELISA) (12,20). Laboratory confirmation for the two cutaneous cases with skin biopsies analyzed in this

Table 4. Results of laboratory testing on seven clinical samples in which 16S type 6 was identified

	Patient		Clinical specimens		
ID[a]	Diagnosis	Laboratory confirmation[b]	Type	Culture	*Bacillus anthracis*–specific PCR[b]
2i	Inhalational anthrax	IHC + PCR of pleural fluid; serology	Tissue	Neg	Neg
10i	Inhalational anthrax	*B. anthracis* isolated from blood and pleural fluid	Pleural fluid	Neg	Pos
			Pleural fluid	Neg	Pos
			Blood	Neg	ND
			Lymph node	Neg	Pos
11i	Inhalational anthrax	*B. anthracis* isolated from blood	Lymph node	Neg	Pos
7c	Cutaneous anthrax	IHC + PCR on skin biopsy	Skin from forehead	Neg	Pos

[a]Patient identification numbers are described in references 12 and 13; I, inhalational case; C, cutaneous case; PCR, polymerase chain reaction.
[b]The immunohistochemical (IHC), serologic, and PCR results are described in reference 14.

study was indeed achieved by these supportive laboratory tests: one case was confirmed by immunohistochemistry and a reactive anti-protective antigen title (IgG ELISA). For the other case all three supportive laboratory tests were positive.

Previously, strains having <3% difference between their 16S rRNA genes were considered the same species (21). However, differences between 16S rRNA genes for some *Bacillus* species, such as *B. anthracis*, *B. cereus*, and *B. thuringiensis,* are <1% (1). Such small differences (e.g., one base between sequences or partial matches at a single nucleotide position in the 16S rRNA gene) have not been used for species differentiation. Our study clearly demonstrates that such small differences might be important for species identification. DNA-DNA hybridization and 16S rRNA sequencing studies have shown that these three *Bacillus* species are closely related and probably represent a single species (3,6,7). If the three were classified as a single species, 16S rRNA sequencing appears to have the potential to differentiate strains at the subspecies level.

Although pXO1 and pXO2 plasmids must be detected to confirm the virulence of *B. anthracis*, 16S rRNA sequencing has a powerful capacity to rapidly identify *B. anthracis* and other species. Although further studies are needed to fully evaluate 16S sequencing as a diagnostic assay, its value as a tool for rapid initial screening in outbreak investigations has been demonstrated.

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References

1. Thorne CB. In: Sonenshein AL, Hock JA, Losick R. *Bacillus subtilis* and other gram-positive bacteria. Washington: American Society for Microbiology; 1993. p. 113–24.
2. Ash C, Farrow JAE, Wallbanks S, Collins MD. Phylogenetic heterogeneity of the *Bacillus* revealed by comparative analysis of small subunit ribosomal RNA sequences. Lett Appl Microbiol 1991;13:202–6.
3. Ash C, Farrow JA, Dorsch M, Stackebrandt E, Collins MD. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. Int J Syst Bacteriol 1991;41:343–6.
4. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, et al. *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*—one species on the basis of genetic evidence. Appl Environ Microbiol 2000;66:2627–30.
5. Kaneko T, Nozaki R, Aizawa K. Deoxyribonucleic acid relatedness between *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis*. Microbiol Immunol 1978;22:639–41.
6. Seki T, Chung C, Mikami H, Oshima Y. Deoxyribonucleic acid homology and taxonomy of the genus *Bacillus*. Int J Syst Bacteriol 1978;28:182–9.
7. Somerville HJ, Jones ML. DNA competition studies within the *Bacillus cereus* group of bacilli. J Gen Microbiol 1972;73:257–65.
8. Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. J Bacteriol 2000;182:2928–36.
9. Khan AS, Morse S, Lillibridge S. Public-health preparedness for biological terrorism in the USA. Lancet 2000;356:1179–82.
10. Logan NA, Turnbull PCB. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of clinical microbiology. 7th ed. Washington: American Society for Microbiology; 1999. p. 357–69.
11. Eden PA, Schmidt TM, Akemore RP, Pace NR. Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. Int J Syst Bacteriol 1991;41:324–5.
12. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
13. Barakat LA, Quentzel HL, Jernigan JA, Kirschke DL, Griffith K, Spear SM, et al. Fatal inhalational anthrax in a 94-year-old Connecticut woman. JAMA 2002;287:863–8.
14. Hoffmaster AR, Meyer RF, Bowen M, Marston CK, Weyant RS, Barnett GA, et al. Evaluation and validation of a real-time PCR assay for rapid identification of *Bacillus anthracis*. Emerg Infect Dis 2002;8:1111–6.
15. Ticknor LO, Kolsto AB, Hill KK, Keim P, Laker MT, Tonks M, et al. Fluorescent amplified fragment length polymorphism analysis of Norwegian *Bacillus cereus* and *Bacillus thuringiensis* soil isolates. Appl Environ Microbiol 2001;67:4863–73.

16. Nubel U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, et al. Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. J Bacteriol 1996;178:5636–43.
17. Rainey FA, Ward-Rainey NL, Janssen PH, Hippe H, Stackebrandt E. *Clostridium paradoxum* DSM 7308T contains multiple 16S rRNA genes with heterogeneous intervening sequences. Microbiology 1996;142:2087–95.
18. Liefting LW, Andersen MT, Beever RE, Gardner RC, Forster RL. Sequence heterogeneity in the two 16S rRNA genes of *Phormium* yellow leaf phytoplasma. Appl Environ Microbiol 1996;62:3133–9.
19. Mylvaganam S, Dennis PP. Sequence heterogeneity between the two genes encoding 16S rRNA from the halophilic archaebacterium *Haloarcula marismortui*. Genetics 1992;130:399–410.
20. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–93.
21. Stackebrandt E, Goebel BM. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species in bacteriology. Int J Syst Bacteriol 1994;44:846–9.

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Antimicrobial Postexposure Prophylaxis for Anthrax: Adverse Events and Adherence

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We collected data during postexposure antimicrobial prophylaxis campaigns and from a prophylaxis program evaluation 60 days after start of antimicrobial prophylaxis involving persons from six U.S. sites where *Bacillus anthracis* exposures occurred. Adverse events associated with antimicrobial prophylaxis to prevent anthrax were commonly reported, but hospitalizations and serious adverse events as defined by Food and Drug Administration criteria were rare. Overall adherence during 60 days of antimicrobial prophylaxis was poor (44%), ranging from 21% of persons exposed in the Morgan postal facility in New York City to 64% of persons exposed at the Brentwood postal facility in Washington, D.C. Adherence was highest among participants in an investigational new drug protocol to receive additional antibiotics with or without anthrax vaccine—a likely surrogate for anthrax risk perception. Adherence of <60 days was not consistently associated with adverse events.

Bioterrorist attacks involving the use of *Bacillus anthracis* in the fall of 2001 caused 22 cases of cutaneous and inhalational anthrax and placed many more persons at risk for this disease because of workplace exposures (1). The massive public health response to these events included an unprecedented prevention program in which approximately 10,000 persons across the eastern United States were offered >60 days of postexposure antimicrobial prophylaxis to prevent inhalational anthrax (2). We describe the exposed population and the provision of postexposure antimicrobial prophylaxis and analysis of data for associated adverse events and adherence to prophylaxis.

The large-scale use of antimicrobial prophylaxis to prevent anthrax within the setting of a bioterrorist attack has never been reported. While ineffective in killing B. anthracis spores, antibiotics are effective against replicating bacteria that develop from the spore following germination. After being inhaled, B. anthracis spores may not germinate immediately but can remain dormant in the lung and lymphatic system for weeks to months as they are slowly cleared by the immune system. As long as spores remain in the body, the risk of germination, replicating B. anthracis, and clinical anthrax exists. Based on initial risk assessments and the estimated efficacy of prophylaxis, antimicrobial postexposure prophylaxis was recommended during the 2001 anthrax outbreak (3,4).

Public health and military officials involved in bioterrorism preparedness initiatives had identified antimicrobial agents of choice for this purpose before the 2001 outbreak (5). Largely through the efforts of these officials, ciprofloxacin and doxycycline were approved by the Food and Drug Administration in 2000 and 2001, respectively, for use as antimicrobial prophylaxis to prevent anthrax and were offered as first-line agents to exposed persons (6,7). Because of safety concerns over the use of ciprofloxacin and doxycycline, amoxicillin, to which B. anthracis is known to be susceptible (8), was offered as prophylaxis to infants, children, and breastfeeding mothers, although it is not approved by the Food and Drug Administration for this indication (2).

In 2001, as soon as the risk for inhalational anthrax was identified, announcements were made recommending antimicrobial prophylaxis to exposed groups at risk; persons in these exposed groups were instructed to obtain prophylaxis from a central distribution point, where antibiotics were supplied from the National Pharmaceutical Stockpile (9). In December 2001, as vaccine became available, the Centers for Disease Control and Prevention offered persons who were recommended for 60 days of antimicrobial prophylaxis the opportunity to receive 40 additional days of antibiotics (ciprofloxacin, doxycycline, or amoxicillin), with or without three doses of anthrax vaccine, through an investigational new drug (IND) protocol. Exposed persons were encouraged to consult with their physicians regarding their individual risk for anthrax and the benefits of participation in the IND protocol (10).

Methods

Antimicrobial prophylaxis campaigns were centered in six sites where persons were exposed: American Media, Inc. employees and visitors in Palm Beach County, Florida; work-

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ers and visitors at the United States Postal Service Trenton Processing and Distribution Center in Hamilton Township, New Jersey; employees and visitors at specific parts of the Hart Senate Office Building in Washington, D.C., as well as congressional mail workers who handled mail for that site; employees and visitors at the Brentwood postal facility in Washington, D.C.; employees working in selected areas of the Morgan postal facility in New York City; and workers and visitors with exposure to the Wallingford and Seymour postal facilities in Connecticut (2). Also among the cohort recommended for at least 60 days of antimicrobial prophylaxis were employees and visitors of the Department of State Annex 32 mailroom facility in Sterling, Virginia, and media workers associated with cutaneous cases in New York City. Ciprofloxacin was initially provided to all persons unless a specific contraindication existed. At the first and second refill visits at the New York City, New Jersey, Brentwood, and Connecticut sites (after antimicrobial susceptibility testing results were available), persons who had been taking ciprofloxacin were encouraged to change to doxycycline, provided no contraindications to doxycycline existed. Persons at the Hart Senate Building were provided a 60-day supply of ciprofloxacin during the first week that antimicrobial prophylaxis was distributed. In Florida, doxycycline was primarily provided at 30-day refill. At all sites, amoxicillin was provided to pregnant women, breastfeeding mothers, children, and some persons who had adverse events associated with ciprofloxacin and doxycycline.

Data Collection

At each site, we used questionnaires distributed primarily at 10- and 30-day refill clinics to collect demographic, clinical, and adherence information. An adverse event was defined as any self-reported symptom while on antimicrobial prophylaxis. Respondents were asked to identify the antimicrobial agent taken most recently and select symptoms experienced while taking this agent from a list of possible adverse events. Early questionnaires used in the first antimicrobial prophylaxis campaign in Florida focused on the presence of a few specific symptoms and medical attention sought for adverse events. Later, in conjunction with 10-day refill clinics, a standardized questionnaire administered at the New Jersey, New York City, and Brentwood facilities collected information on a broader list of adverse events. We did not analyze 10-day New York City data because a large number of persons completed the questionnaires who had discontinued postexposure prophylaxis as recommended at 10-day follow-up. Modified versions of this initial questionnaire were used at 30 days at the Florida, New Jersey, Hart Senate Building, Brentwood, and New York City facilities. Questionnaires were self-administered in all sites except New Jersey, where they were administered by a health-care worker.

Potentially serious adverse events were identified based on adverse event data collected at 10- and 30-day follow-up (11,12). Persons who reported seeking medical attention because of adverse events associated with antimicrobial prophylaxis were further investigated. The definition of a serious adverse event, based on the Code of Federal Regulations (21 CFR 314.80), was applied to any of the following events associated with antimicrobial prophylaxis: death, life-threatening adverse event, inpatient hospitalization or prolongation of an existing hospitalization, persistent or substantial disability/incapacity, congenital anomaly/birth defect, or an important medical event that requires medical or surgical intervention to avert one of these outcomes. A clinician interviewed health-care providers and reviewed medical charts to assess the severity of the adverse events and determine whether they met the case definition. The relationship of the adverse event to the antimicrobial agent used was categorized as definite, probable, possible, remote, not related, and cannot assess. At day 30, a standardized data collection form was used.

Program Evaluation after 60 Days

Beginning in late January 2002, after persons at each site had completed at least 60 days of antimicrobial prophylaxis, we evaluated the program for all persons in the exposed cohort. In our analysis, we included only persons who stated that they were recommended for at least 60 days of prophylaxis during the program evaluation interview. Through brief telephone interviews using a standardized questionnaire, we collected information on the ability of exposed persons to obtain antimicrobial prophylaxis and informational materials, associated adverse events, and adherence to prophylaxis. Adherence was defined as self-reported use of antimicrobial prophylaxis for at least 60 days. Respondents indicating the presence of adverse events were asked to identify their most severe or "single most serious" symptom, then identify other associated symptoms from a list of potential adverse events. Adverse events identified after the 60-day follow-up could be associated with overall use of antimicrobial prophylaxis, meaning respondents were attributing adverse events to one or more agents used as antimicrobial prophylaxis. Measures of perceived severity of symptoms, including whether medical attention was sought for adverse events, were included. Persons reporting nonadherence were asked to give the most important reason for not taking the antibiotic. We made multiple attempts to reach identified persons; follow-up to determine characteristics of nonrespondents is ongoing. Investigation of potentially serious adverse events reported after the 60-day follow-up is planned in a manner similar to prior serious adverse event evaluations.

Data Analysis

Statistical analyses were conducted by using SAS version 8 (SAS Institute, Inc., Cary, NC) statistical software. We used the c^2 test to compare proportions across each of the six sites; $p<0.05$ was considered statistically significant.

We conducted two separate analyses for each of the six sites after 60 days using program evaluation data: one for nonadherence and one for occurrence of adverse events. The dependent (outcome) variable for the first analysis was

nonadherence (nonadherence [1–59 days of antimicrobial prophylaxis] versus adherence [≥60 days of antimicrobial prophylaxis]). The dependent (outcome) variable for the adverse event analysis was self-reported adverse events (a symptom reported versus no symptom reported). We excluded persons who reported not obtaining their prophylaxis or not taking any of it, as well as those for whom adherence information was not available. We constructed a logistic regression model for each dependent variable for each of the six sites. The independent (predictor) variables used in the logistic models included demographic and clinical variables from the 60-day program evaluation, including gender, age group, race, ethnicity, presence of adverse events, and participation in the IND protocol. Independent variables were retained in each of the site-specific models if their estimated parameters were statistically significant ($p<0.05$) in any model for the same dependent variable. We assessed colinearity and two-way interactions for all variables in each of the final multivariable models.

Results

Approximately 10,000 persons were recommended for at least 60 days of antimicrobial prophylaxis to prevent inhalational anthrax. The largest number of persons on antimicrobial prophylaxis was associated with the Brentwood facility (n=2,743) and the smallest with the Hart Senate Building (n=600). We completed interviews on 6,178 persons; participation rates varied by site (Table 1).

Most of the respondents were 40–64 years of age, and 60% were men. Of 2,444 women, 2% reported being pregnant or having been pregnant while taking antimicrobial prophylaxis. Median age was lowest at the Hart Senate Building site and highest at the Brentwood facility. Approximately 150 persons were <18 years of age at the start of the antimicrobial prophylaxis campaign; the Florida site had the most children (n=88). The number of children was estimated based on data collected at 10 and 30 days. Persons <18 years were not interviewed as part of the program evaluation after 60 days. Forty-one percent of respondents reported their race as white and 42% as African-American, but marked variation existed by site. Members of the Florida and Hart Senate Building cohorts were primarily Caucasian, while persons at Brentwood facility were primarily African-American (Table 2).

Almost all (97%) respondents obtained an initial supply of antimicrobial prophylaxis. Three percent (n=182) of respondents reported difficulty in obtaining their supply of prophylaxis, and of these, most (83%) were able to get 60 days of prophylaxis. Ten percent of respondents took no antimicrobial prophylaxis, although they collected an initial supply. This group and those who never obtained antimicrobial prophylaxis compose the overall group of 787 respondents who reported not taking any of their prophylaxis. Forty-eight respondents did not provide any adherence information. Persons who took at least one dose of antimicrobial prophylaxis numbered 5,343 (86%); fewer than half of these respondents took only one agent as antimicrobial prophylaxis. Fifty-nine percent of respondents taking at least one dose of antimicrobial prophylaxis (n=3,156) took two antimicrobial agents as prophylaxis; 56% (n=2,984) took ciprofloxacin for one part of their course and doxycycline for the rest. Data from 10, 30, and post-60 days show an overall shift in the most recent antimicrobial agent used from ciprofloxacin (84% at day 10) to doxycycline (61% at day 60).

Table 1. Response rates for persons recommended for at least 60 days of postexposure antimicrobial prophylaxis, 2001–2002

Anthrax investigation site	No. of persons prescribed prophylaxis[a]	Response rates for prophylaxis		
		10 days (%)	30 days (%)	60 days[b,c] (%)
Florida	1,082	81	40	78
New Jersey	1,402	25	64	76
Hart Senate Building, Washington, D.C.	600	n/a[d]	59	82
Brentwood facility, Washington, D.C.	2,743	60	45	62
New York City	2,259	n/a	23	58
Connecticut	1,217	n/a	n/a	69

[a]When determining the number of persons prescribed prophylaxis, we excluded program evaluation respondents who indicated that they were not recommended for ≥60 days of antimicrobial prophylaxis. This number may vary from estimates using other data.
[b]Lists of persons in groups recommended for ≥60 days of antimicrobial prophylaxis were sometimes available only later in the campaign, so denominators may vary slightly for each collection period.
[c]No 60-day follow-up available on persons <18 years of age at time of 60-day evaluation.
[d]n/a, not available.

Adverse Events

Of the 5,343 persons who reported taking at least one dose of antimicrobial prophylaxis, 57% (n=3,032) reported adverse events during the first 60 days of antimicrobial prophylaxis use. Reporting of adverse events varied by site, ranging from 42% of respondents at the Connecticut facility to 65% at the Brentwood facility. Thirty-two percent of respondents with adverse events reported diarrhea or stomach pain with their most recent antibiotic, 27% nausea or vomiting, 25% headache, and 22% dizziness. The most commonly reported categories of symptoms were gastrointestinal (44%, including nausea or vomiting, diarrhea or stomach pain, heartburn, and pain with swallowing) and neurologic (33%, including headache, dizziness, lightheadedness, fainting, and seizure). Of the 3,032 persons reporting at least one adverse event, 23% identified "diarrhea or stomach pain" and 19% "nausea or vomiting" as their "most serious" symptom. Among persons reporting adverse events, 14% graded them as severe, 45% as moderate, and 41% as none/mild. Twenty-six percent of persons with adverse events reported missing at least 1 day of work because of symptoms.

At 10 days, the rate of one or more adverse events among persons taking ciprofloxacin most recently (45%) did not differ significantly from that of persons taking doxycycline

Table 2. Demographic data for persons recommended for at least 60 days of postexposure prophylaxis, 2001–2002

Characteristic	Florida (%) (n=780)	New Jersey (%) (n=1,061)	Hart Senate Building, Washington, D.C. (%) (n=485)	Brentwood facility, Washington, D.C. (%) (n=1,694)	New York City (%) (n=1,315)	Connecticut (%) (n=843)
Male	62	66	59	58	55	67
Pregnant	2	1	3	2	2	3
Caucasian	84	63	76	5	17	63
African-American	4	23	16	87	45	21
Median age (yrs)	40	46	34	51	46	44
Age range (yrs)	(17–86)	(18–77)	(17–75)	(19–79)	(18–78)	(17–85)

most recently (49%). At day 30, this rate was slightly higher (77%) among persons taking ciprofloxacin most recently than persons taking doxycycline most recently (71%, $p<0.01$) (Table 3).

Univariate analysis of factors associated with the presence of adverse events showed male respondents were less likely to report adverse events than were female respondents in all sites except Connecticut. Compared with the youngest age group, persons who reported adverse events were less likely to be ≥65 years of age. Persons with adverse events were significantly more likely to enroll in the IND protocol in the Brentwood facility (Table 4).

Multivariable analysis also showed that persons reporting adverse events were less likely to be male in all sites except Connecticut. At the Hart Senate Building site, persons with adverse events were less likely to be African-American. At the Brentwood site, persons with adverse events were more likely to have enrolled in the IND protocol.

Medical Attention for Adverse Events and Serious Adverse Events

Of 2,907 persons participating in 10-day follow-up, 7% reported seeking medical attention. Follow-up at 10 days for serious adverse events in the Florida, New Jersey, and New York City facilities found no hospitalizations attributable to antimicrobial prophylaxis in persons seeking medical care for symptoms consistent with anaphylaxis (difficulty breathing, rash or itchy skin, throat tightness, or lip and tongue swelling) (11). Of 3,374 persons participating in 30-day follow-up, 13% reported seeking medical attention. Of 2,135 persons with follow-up information available at 30 days in the Florida, New Jersey, New York City, and the Hart Senate Building facilities, seven persons (0.3%) were found to have had a serious adverse event, including three persons hospitalized. Ten- and 30-day follow-up data were not available for Connecticut. Four persons had reactions in which the relationship to antimicrobial prophylaxis was judged to be definite or probable, while the remaining three were classified as not related or could not assess. Two of four serious adverse events with a definite or probable relationship to antimicrobial prophylaxis were characterized by diffuse rash and systemic symptoms; the remaining two involved swelling of the face and neck. Two persons were treated as outpatients, one was treated in the emergency department, and the remaining patient was briefly hospitalized. All four recovered without sequelae.

At the post 60-day evaluation, 16% of respondents who took at least one dose of antimicrobial prophylaxis (n=842) reported seeking medical care for adverse events caused by prophylaxis at some time during their 60-day course. Nine percent (n=493) reported that their physician or other health-care provider advised them to stop taking antibiotics; 54% of these persons (n=267) reported that the presence of adverse events was the only reason for the recommendation to discontinue. Medical follow-up of persons reporting potentially serious adverse events after 60 days is ongoing.

Table 3. Adverse events at 10 and 30 days, by most recent antimicrobial agent, all sites,[a] 2001–2002

	Day 10			Day 30		
Adverse events	Ciprofloxacin (%) (n=2,446)	Doxycycline (%) (n=165)	p value	Ciprofloxacin (%) (n=737)	Doxycycline (%) (n=2,050)	p value
≥1 adverse event	45	49	0.27	77	71	<0.01
Gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain, or heartburn)	26	26	0.89	42	49	<0.01
Fainting, dizziness, light-headedness, or seizures[b]	18	11	0.08	23	18	0.01
Rash, hives, or itchy skin	7	7	0.8	14	14	0.6
Joint problems[b]	8	7	0.6	25	16	<0.01

[a]Day 10 data includes the Florida, New Jersey, and Washington, D.C., Brentwood sites; Day 30 data includes the Florida, New Jersey, Washington, D.C., Hart Senate Building , and New York City sites.
[b]Day 10 reports of these symptoms not collected at the Florida site.

Table 4. Univariate analysis of factors associated with adverse events, post 60-day program evaluation data, 2001–2002[a]

	Reports of adverse events among persons taking at least one dose of prophylaxis											
	Florida (n=744)		New Jersey (n=1,028)		Hart Senate Building, Washington, D.C. (n = 472)		Brentwood facility, Washington, D.C. (n=1,619)		New York City (n=882)		Connecticut (n=598)	
Variable	(%)	p value	(%)	p value	(%)	p value	(%)	p value	(%)	p value	(%)	p value
Gender												
Male	50	<0.01	54	<0.01	54	0.01	60	<0.01	44	<0.01	41	0.53
Female	63	Ref	68	Ref	65	Ref	74	Ref	61	Ref	44	Ref
Age group												
17–39 yr	55	Ref	58	Ref	61	Ref	69	Ref	56	Ref	40	Ref
40–64 yr	55	0.99	59	0.75	53	0.10	66	0.38	51	0.20	44	0.37
≥65 yr	41	0.19	39	0.03	40	0.34	52	0.04	37	0.04	19	0.10
Race												
African-American	54	0.88	61	0.35	46	0.02	67	0.36	52	0.74	36	0.14
All others	55	Ref	58	Ref	61	Ref	63	Ref	51	Ref	44	Ref
Ethnicity												
Hispanic	55	1.00	60	0.87	44	0.38	57	0.38	56	0.12	39	0.65
Non-Hispanic	55	Ref	59	Ref	59	Ref	66	Ref	50	Ref	42	Ref
IND enrollment												
Yes	48	0.29	61	0.34	68	0.11	71	<0.01	56	0.40	50	0.13
No	56	Ref	57	Ref	57	Ref	63	Ref	51	Ref	41	Ref
Received printed materials about adverse events												
No	70	0.03	61	0.73	73	<0.01	78	0.02	29	0.01	53	0.36
Yes	54	Ref	58	Ref	54	Ref	66	Ref	53	Ref	42	Ref

[a]IND, investigational new drug; Ref, referent.

Adherence

Fewer than half of respondents (44%, n=2,712) reported taking antimicrobial prophylaxis for at least 60 days. Adherence through 60 days was highest at the Brentwood facility (64%) and lowest at the New York City facility (21%) (Figure). Of persons who took at least one dose of antimicrobial prophylaxis, 72% (n=3,873) reported taking their medicine daily as prescribed, and 19% (n=1,027) reported taking prophylaxis "almost every day." Eighty-six percent of all respondents were aware of the IND.

Of 2,631 persons taking at least one dose of antimicrobial prophylaxis but stopping before 60 days, 43% stated that adverse events were the most important reason they discontinued prophylaxis, 25% reported perception of a low risk for anthrax, and 7% identified fear of long-term side effects from antimicrobial prophylaxis. Of the 172 who never obtained their prophylaxis, 54% reported perception of a low personal risk for anthrax as the most important reason for not obtaining the recommended antimicrobial agent.

On univariate analysis, in some sites nonadherent respondents were more likely to be African-American, and in other sites they were more likely to be Hispanic. In New York City, nonadherent persons were more likely to have sought medical care and were more likely to have been advised by a healthcare provider to stop taking their antimicrobial prophylaxis. In all sites, respondents who enrolled in the IND were more

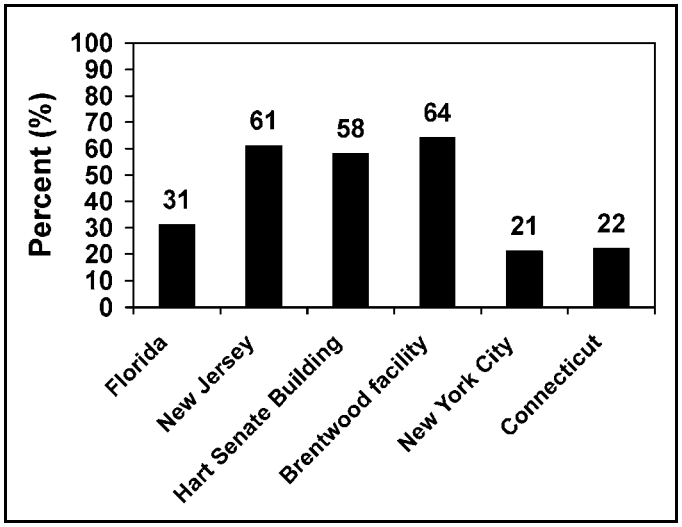

Figure. Percentage of persons completing at least 60 days of antimicrobial prophylaxis, by U.S. site, 2001–2002.

likely to have been adherent. These associations were statistically significant in all but one site (Connecticut) (Table 5).

Six site-specific logistic regression models showed an adverse event to be associated with <60 days' adherence in two sites only (Florida and New York City). Respondents who enrolled in the IND were more likely to have been adherent in all sites except Connecticut. Hispanic persons were more likely to be nonadherent in New Jersey and New York City; African-American persons were more likely to be nonadherent in New Jersey and the Hart Senate Building site. Persons in the 40- to 64-year age group were less likely to be nonadherent in the Florida, New Jersey, Brentwood, and New York City sites (Table 6).

Discussion

The anthrax outbreak of 2001 represents the first bioterrorist attack in the United States using *B. anthracis* and the first recorded mass postexposure antimicrobial prophylaxis campaign to prevent inhalational anthrax. Monitoring for adverse events and adherence during this campaign offers a unique opportunity to evaluate associated adverse events and adherence to antimicrobial agents in a mass prophylaxis campaign.

Table 5. Univariate analysis of factors associated with nonadherence, post 60-day program evaluation data, 2001–2002[a]

	Reports of nonadherence[b] among persons who took at least one dose of postexposure prophylaxis											
	Florida (n=744)		New Jersey (n=1,028)		Hart Senate Building, Washington, D.C. (n = 472)		Brentwood facility, Washington, D.C. (n=1,619)		New York City (n=882)		Connecticut (n=598)	
Variable	(%)	p value	(%)	p value	(%)	p value	(%)	p value	(%)	p value	(%)	p value
Gender												
Male	67	0.57	32	<0.01	39	0.71	32	0.27	62	<0.01	68	0.68
Female	69	Ref	47	Ref	41	Ref	34	Ref	77	Ref	70	Ref
Age group												
17–39 yr	75	Ref	53	Ref	42	Ref	52	Ref	80	Ref	71	Ref
40–64 yr	61	<0.01	32	<0.01	37	0.33	29	<0.01	67	<0.01	68	0.62
≥65 yr	64	0.23	26	<0.01	0	0.08	29	<0.01	33	<0.01	31	<0.01
Race												
African-American	82	0.10	44	0.02	53	0.02	33	0.83	72	0.15	72	0.37
All others	67	Ref	35	Ref	38	Ref	32	Ref	67	Ref	68	Ref
Ethnicity												
Hispanic	65	0.52	60	<0.01	60	0.19	29	0.67	76	0.03	75	0.30
Non-Hispanic	68	Ref	37	Ref	40	Ref	33	Ref	67	Ref	67	Ref
Adverse events												
Yes	75	<0.01	38	0.40	39	0.72	32	0.49	75	<0.01	72	0.13
No	59	Ref	36	Ref	41	Ref	34	Ref	63	Ref	66	Ref
Severity of adverse events												
None	64	Ref	37	Ref	45	Ref	33	Ref	63	Ref	65	Ref
Mild	66	0.58	29	0.07	34	0.07	27	0.06	65	0.58	63	0.73
Moderate/severe	72	0.05	44	0.09	43	0.66	36	0.38	79	<0.01	76	0.01
IND enrollment												
Yes	46	<0.01	19	<0.01	7	<0.01	16	<0.01	49	<0.01	60	0.08
No	70	Ref	46	Ref	46	Ref	43	Ref	72	Ref	70	Ref
Missed ≥1 day of work												
Yes	72	0.47	40	0.28	33	0.39	32	0.44	77	0.03	74	0.23
No	68	Ref	36	Ref	41	Ref	34	Ref	67	Ref	67	Ref
Sought medical attention												
Yes	72	0.36	40	0.42	34	0.18	32	0.58	86	<0.01	65	0.62
No	67	Ref	37	Ref	42	Ref	33	Ref	67	Ref	69	Ref

[a] IND, investigational new drug.
[b] 1–59 days of postexposure prophylaxis.

Table 6. Factors associated with nonadherence, multivariable analysis, post 60-day data, 2001–2002[a]

Variable	Participants reporting non-adherence (1–59 days of antimicrobial prophylaxis) among persons taking at least one dose					
	Florida (n=744) OR (95% CI)	New Jersey (n=1,028) OR (95% CI)	Hart Senate Building (n=472) OR (95% CI)	Brentwood facility (n=1,619) OR (95% CI)	New York City (n=882) OR (95% CI)	Connecticut (n=598) OR (95% CI)
Gender						
Male	1.00 (0.71, 1.41)	0.56 (0.42, 0.76)	0.92 (0.61, 1.38)	0.90 (0.71, 1.13)	0.57 (0.41, 0.79)	0.99 (0.67, 1.46)
Female	Referent	Referent	Referent	Referent	Referent	Referent
Age						
17–39 yr	Referent	Referent	Referent	Referent	Referent	Referent
40–64 yr	0.51 (0.36, 0.72)	0.54 (0.40, 0.73)	0.82 (0.53, 1.28)	0.42 (0.32, 0.56)	0.61 (0.41, 0.90)	0.96 (0.64, 1.44)
≥65 yr	0.73 (0.28, 1.89)	0.51 (0.23, 1.12)	n/a	0.49 (0.24, 1.02)	0.17 (0.08, 0.39)	0.21 (0.07, 0.65)
Race						
African-American	1.80 (0.66, 4.92)	1.47 (1.06, 2.05)	1.87 (1.08, 3.25)	0.90 (0.63, 1.28)	1.08 (0.76, 1.52)	1.39 (0.86, 2.24)
All others	Referent	Referent	Referent	Referent	Referent	Referent
Ethnicity						
Hispanic	0.78 (0.47, 1.29)	2.42 (1.21, 4.86)	1.58 (0.42, 6.01)	0.62 (0.23, 1.69)	1.57 (1.03, 2.39)	1.42 (0.68, 2.93)
Non-Hispanic	Referent	Referent	Referent	Referent	Referent	Referent
Adverse events						
Yes	2.23 (1.61, 3.09)	1.08 (0.82, 1.44)	1.07 (0.71, 1.62)	0.98 (0.77, 1.24)	1.58 (1.16, 2.16)	1.32 (0.92, 1.90)
No	Referent	Referent	Referent	Referent	Referent	Referent
IND enrollment						
Yes	0.36 (0.21, 0.64)	0.27 (0.19, 0.38)	0.09 (0.04, 0.24)	0.28 (0.22, 0.36)	0.33 (0.21, 0.52)	0.64 (0.39, 1.05)
No	Referent	Referent	Referent	Referent	Referent	Referent

[a]OR, odds ratio; CI, confidence interval; IND, investigational new drug.

Our data show that the rate of serious adverse events was low, and adverse event monitoring to date has shown no deaths due to antimicrobial prophylaxis. Mild adverse events or adverse events that did not fulfill criteria as serious were common, and adherence to recommendations for at least 60 days of antimicrobial prophylaxis was poor.

The overall rate of reported adverse events during this campaign was higher than the rate (16.5%) listed on the usage information provided with ciprofloxacin (13). (The information provided for doxycycline does not include a rate for adverse events, so a similar comparison cannot be made with this agent.) However, comparison of these rates with adverse event rates associated with antimicrobial prophylaxis must be made with caution. Adverse events reported in the ciprofloxacin literature are categorized by their likelihood to be drug related, while this relationship was assessed only for the small proportion of potentially serious adverse events resulting from antimicrobial prophylaxis. Adverse event rates are ideally derived from data collected under controlled circumstances, including the presence of a control group, while these data were collected as part of a response to a public health emergency. Published adverse event rates among patients taking ciprofloxacin or doxycycline in clinical settings where a similar definition of adverse event is used provide a closer comparison of adverse event rates to antimicrobial prophylaxis. A recent published review of adverse events among patients taking long-term (>30 days) ciprofloxacin in clinical trials found an overall rate of 32% and a rate of gastrointestinal adverse events of 22% (14). In several small studies, the rate of adverse events among patients on doxycycline has been shown to be >30% and as high as 50%, with rates of nausea and vomiting of 31%, depending on the reporting method used (15–20).

Adverse events to antimicrobial prophylaxis in this event may be attributed to the known pharmacology of the drugs taken. However, some portion of the adverse events may also be ascribed to above-average symptom awareness related to fear of contracting anthrax. Data from focus groups of exposed workers support this hypothesis and suggest that self-reports of stress were frequent (21). Anxiety may have led to symptoms or physiologic changes that cannot be explained on the basis of the known pharmacology of the antimicrobial agents given but are temporally related to antimicrobial prophylaxis (22). Regardless of their relation to antimicrobial prophylaxis or fulfillment of criteria for serious adverse events, high rates of reported adverse events during this event suggest the need for a management strategy in addition to monitoring efforts for future antimicrobial prophylaxis campaigns.

While overall adverse events rates were high, differences in rates of adverse events associated with ciprofloxacin compared with those associated with doxycycline were not sub-

stantial. Many exposed persons were encouraged to change from ciprofloxacin to doxycycline midway through their course for reasons not related to adverse events (23). Because more than half of persons switched from ciprofloxacin to doxycycline or vice versa, attribution of adverse events to a specific antimicrobial agent is possible only with data collected at the first and second refill visits; adverse event data collected at the program evaluation after 60 days reflect overall adverse events to antimicrobial prophylaxis. Nonetheless, available agent-specific adverse event data do not show differences between ciprofloxacin and doxycycline of the magnitude to warrant preference for one agent over the other in a future antimicrobial prophylaxis campaign.

Overall adherence to recommendations to take at least 60 days of antimicrobial prophylaxis was poor. While adherence to any medical treatment or prophylaxis regimen is essential for treatment to be successful, adherence to antimicrobial prophylaxis is thought to be particularly important because of the risk among persons exposed to *B. anthracis* aerosols for developing anthrax while spores are slowly cleared from lung and thoracic lymphatic systems (4). For this analysis we chose premature discontinuation of antimicrobial prophylaxis as a surrogate for nonadherence, although errors in amount, timing, or frequency can also constitute nonadherence to a medication regimen (24). We found the factor most consistently associated with adherence to be IND participation, which we interpret as a surrogate for perception of individual risk. Because exposed persons were asked to consider their risk for anthrax and the guidance of their health-care provider when making their decision, IND participation is a marker for an person's perception of risk for inhalational anthrax. Some of the respondents who perceived their risk for anthrax to be high may have been reluctant to enroll in the IND at the end of the initial 60-day regimen because of adverse events in response to antimicrobial prophylaxis, but our univariate analysis did not demonstrate that persons with adverse events were less likely to enroll in the IND protocol. The strong association between risk perception and adherence to antimicrobial prophylaxis is consistent with previous studies of a variety of health conditions, which have demonstrated that effective risk communication based on a close patient-provider relationship is a crucial determinant of adherence (24–26).

The presence of adverse events was not consistently associated with nonadherence on univariate and multivariable analysis. When asked directly, a higher proportion of nonadherent respondents indicated that the most important reason for their premature discontinuation was adverse events, rather than a low personal risk for anthrax (43% vs. 25%, $p<0.01$). Of the 1,120 respondents who reported discontinuing antimicrobial prophylaxis because of adverse events, at another point in the interview 16% said that they did not have any adverse events. Despite the fact that many persons recall discontinuing antimicrobial prophylaxis because of adverse events, our analysis showed that risk perception is a stronger and more consistent predictor of adherence across the six exposed cohorts.

Data on adverse events and adherence must be interpreted in light of the unusual circumstances of the bioterrorist attacks of 2001. The difference in clinical and demographic variables between the six sites prevented us from identifying factors related to nonadherence or adverse events for the exposed cohort as a whole. In any future bioterrorist-related *B. anthracis* exposure, site-specific circumstances of the attack and the nature of the exposed population must be taken into account during antimicrobial prophylaxis campaigns. Future adherence promotion activities should consider existing theoretical models developed to predict health behaviors, which often stress the importance of understanding persons' interest and concern about their health, their perception of the level of risk to their health, and education regarding the consequences of the health problem (27). Adverse event management efforts should help exposed persons manage adverse events regardless of whether they are serious or related to antimicrobial prophylaxis or the terrorism itself. The threat of bioterrorism remains, and we must incorporate lessons learned from the bioterrorist attacks of 2001 to prepare for any future attacks. The data presented here offer public health decision-makers reassurance regarding the low proportion of serious adverse events to antimicrobial prophylaxis and guidance regarding the expected level of adherence during prophylaxis campaigns. Adherence promotion and adverse events management will be essential components to providing this potentially life-saving intervention.

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References

1. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax—Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1077–9.
2. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1008–10.
3. Friedlander AM, Welkos SL, Pitt ML, Ezzell JW, Worsham PL, Rose KJ, et al. Postexposure prophylaxis against experimental inhalation anthrax. J Infect Dis 1993;167:1239–43.
4. Henderson DW, Peacock S, Belton FC. Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. J Hyg 1956;54:28–36.
5. Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, et al. Anthrax as a biological weapon: medical and public health management. Working group on civilian biodefense. JAMA 1999;281:1735–45.
6. Food and Drug Administration. Prescription drug products; doxycycline and penicillin G Procaine administration for inhalational anthrax (post-exposure). Federal Register 2001;66:55679.
7. Food and Drug Administration. Medical review. Available from: URL: http://www.fda.gov/cder/foi/nda/2000/19-537S038_cipro.htm. Accessed November 2001.
8. Lightfoot NF, Scott RJD, Turnbull PCB. Antimicrobial susceptibility of Bacillus anthracis. Salisbury Medical Bulletin 1990;68:95–8.
9. Centers for Disease Control and Prevention. National pharmaceutical stockpile program. Available from: URL: http://www.cdc.gov/nceh/nps/. Accessed July 3, 2002.
10. Centers for Disease Control and Prevention. Notice to readers: additional options for preventive treatment for persons exposed to inhalational anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1142,1151.
11. Centers for Disease Control and Prevention. Update: adverse events associated with anthrax prophylaxis among postal employees—New Jersey, New York City, and the District of Columbia Metropolitan Area, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1051–4.
12. Shepard CW. Adverse events associated with post-exposure antimicrobial prophylaxis for the prevention of anthrax—Florida, New Jersey, New York City, Maryland, Virginia, and Washington, D.C., 2001. In: 51st Annual Epidemic Intelligence Service Conference program and abstracts book. Atlanta: Centers for Disease Control and Prevention: 2002. p. 51.
13. Physicians Desk Reference. Available from: URL: http://www.pdrel.com. Accessed July 3, 2002.
14. Segev S, Yaniv I, Haverstock D, Reinhart M. Safety of long-term therapy with ciprofloxacin: data of controlled clinical trials and review. Clin Infect Dis 1999;28:299–308.
15. Bryant SG, Fisher S, Kluge RM. Increased frequency of doxycycline side effects. Pharmacotherapy 1987;7:125–9.
16. McCormack WM, Martin DH, Hook EW, Jones RB. Daily oral grepafloxacin vs. twice daily oral doxycycline in the treatment of Chlamydia trachomatis endocervical infection. Infect Dis Obstet Gynecol 1998;6:109–15.
17. Ragnar NS. Atypical pneumonia in Nordic countries: aetiology and clinical results of a trial comparing fleroxacin and doxycycline. J Antimicrob Chemother 1997;39:499–508.
18. Phillips MA, Kass RB. User acceptability patterns for mefloquine and doxycycline malaria chemoprophylaxis. J Travel Med 1996;3:40–5.
19. Raoult D, Houpikian P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P, et al. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Intern Med 1999;159:167–73.
20. Schuhwerk M, Behrens RH. Doxycycline as first line malarial prophylaxis: how safe is it? J Travel Med 1998;5:102.
21. Jefferds MD, Roy S, Hayslett J, Frye A, Laserson K, Schuchat A, et al. Promoting postal workers' adherence to antibiotic prophylaxis to prevent inhalational anthrax— Washington, DC, 2001. In: 51st Annual Epidemic Intelligence Service Conference program and abstracts book. Atlanta: Centers for Disease Control and Prevention: 2002. p. 73.
22. Barsky AJ, Saintfort R, Rogers MP, Borus JF. Nonspecific medication side effects and the nocebo phenomenon. JAMA 2002;287:622–7.
23. Centers for Disease Control and Prevention. Notice to readers: interim guidelines for investigation of and response to *Bacillus anthracis* exposures. MMWR Morb Mortal Wkly Rep 2001;50:987–90.
24. Sanson-Fisher R, Bowman J, Armstrong S. Factors affecting nonadherence with antibiotics. Diagn Microbiol Infect Dis 1992;15:103S–9S.
25. Lam F, Stevenson FA, Britten N, Stell IM. Adherence to antibiotics prescribed in an accident and emergency department: the influence of consultation factors. Eur J Emerg Med 2001;8:181–8.
26. Shea S, Misra D, Ehrlich MH, Field L, Francis CK. Correlates of nonadherence to hypertension treatment in an inner-city minority population. Am J Public Health 1992;82:1607–12.
27. Trick LR. Patient compliance—don't count on it! J Am Optometric Assoc 1993;64:264–70.

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Anthrax Postexposure Prophylaxis in Postal Workers, Connecticut, 2001

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After inhalational anthrax was diagnosed in a Connecticut woman on November 20, 2001, postexposure prophylaxis was recommended for postal workers at the regional mail facility serving the patient's area. Although environmental testing at the facility yielded negative results, subsequent testing confirmed the presence of *Bacillus anthracis*. We distributed questionnaires to 100 randomly selected postal workers within 20 days of initial prophylaxis. Ninety-four workers obtained antibiotics, 68 of whom started postexposure prophylaxis, and of these, 21 discontinued. Postal workers who never started or stopped taking prophylaxis cited as reasons disbelief regarding anthrax exposure, problems with adverse events, and initial reports of negative cultures. Postal workers with adverse events reported predominant symptoms of gastrointestinal distress and headache. The influence of these concerns on adherence suggests that communication about risks of acquiring anthrax, education about adverse events, and careful management of adverse events are essential elements in increasing adherence.

On November 20, 2001, *Bacillus anthracis* was confirmed in blood cultures from a 94-year-old woman in rural Oxford, Connecticut, who was diagnosed with inhalational anthrax and died 1 day later (1,2). No obvious source of exposure to *B. anthracis* was identified. She was the 22nd patient diagnosed with anthrax in the United States in 2001 (3). Before this case, all patients diagnosed with inhalational anthrax had had contact with intentionally contaminated mail delivered through the postal system, with the exception of a patient in New York City (where an investigation was under way). Since the source of transmission was identified as the mail for all but one anthrax case, investigation of area postal facilities began immediately.

The mail was considered a likely source of contamination for the patient in Connecticut, and postexposure antimicrobial prophylaxis was recommended for postal workers employed in the regional distribution center and local post office serving the patient's area. At the regional postal distribution center, which operates 24 h a day and employs 1,122 workers, employees work one of three 8-h shifts and process approximately 3 million pieces of mail daily.

The regional processing center contains 29 high-speed sorting machines. In contrast, the local post office, a two-room structure with 48 employees, has no high-speed sorting machines. All mail collected in the local post office is sent to the regional processing center. The post office serves two zip code areas; mail requiring sorting for the two zip codes is hand-sorted at the local level by carrier route.

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The Connecticut Department of Public Health (CDPH), in consultation with the Centers for Disease Control and Prevention (CDC), recommended postexposure prophylaxis as a precaution to protect the health of the postal workers in these facilities (4). As part of a national distribution center sampling protocol, an independent contractor working for the United States Postal Service (USPS) took environmental samples on November 11, but anthrax spores had not been isolated in the regional distribution center. The decision was made to offer prophylaxis to postal workers pending the results of additional, more focused testing.

The first of many postexposure prophylaxis clinics was held on November 21, 2001. Postal workers were given an initial 10-day course of ciprofloxacin unless contraindicated (5–7). Nasal swabs were collected from the postal workers at the first clinics to determine if contamination was present in the facilities, rather than to diagnose or define individual exposure (8). *B. anthracis* was not isolated from any of 485 nasal swabs taken from postal workers.

On November 21, 25, and 28 and December 2, increasingly focused environmental sampling was performed of both the regional distribution center and the local post office to determine whether any contaminated mail had passed through the facilities (9). Samples obtained on November 21 and 25 were negative; samples taken on November 28 and December 2 from four high-speed sorting machines in the regional distribution center were positive. No contamination was identified in the local post office. Based on the positive results, the CDPH recommended that prophylaxis be extended for a full course of 60 days for all postal workers in the regional facility. Facility management conducted a progressive series of town hall meetings to notify postal employees of the test results at

the various facilities, as well as results of postal worker nasal swabs. Although contaminated sorting machines were shut down for machine-specific decontamination, the regional distribution facility remained open.

Antimicrobial testing of the Connecticut patient's isolates confirmed the sensitivity of this *B. anthracis* strain to both doxycycline and ciprofloxacin. For the continuation phase of prophylaxis, doxycycline was offered as the primary antibiotic unless contraindications existed or the workers specifically requested to continue on ciprofloxacin.

On December 10, 2001, we conducted a survey to evaluate postal workers' adherence to postexposure prophylaxis and to identify factors influencing their degree of adherence. This article describes the findings of the study.

Methods

Of the 1,122 postal workers at the regional distribution center, we randomly selected 100 from the night and day shifts. Five workers declined; five additional workers were randomly selected and agreed to participate (refusal rate 5%). CDC health officials interviewed the group of postal workers using a standardized questionnaire to collect information on demographics, adherence, side effects, and attitudes regarding postexposure prophylaxis and exposure risk. Several characteristics were examined for determinants of starting prophylaxis, including sex, race, and age, as well as whether the postal worker worked on high-speed machinery or obtained an influenza vaccine. For comparison, age was divided into quartiles. The lowest quartile (age ≤37 years) was compared with the top three quartiles, and the highest quartile (age ≥52) was compared with the bottom three quartiles. Serious side effects were defined as those causing death, hospitalization, persistent or substantial disability, or birth defects, or requiring intervention to avoid these outcomes (10). We conducted our analysis using SAS software, version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Of the 100 postal workers sampled, 66% were men. Mean age was 45 years (range 19–65 years). Ethnicities reported were Caucasian (71%), African-American (23%), Asian/Pacific Islander (4%), and Hispanic (2%). None of the respondents were pregnant. Fifteen employees worked on high-speed sorting machines. Forty-two postal workers reported obtaining an influenza vaccine during the previous 3 months.

Ninety-four of the 100 workers surveyed acquired antibiotics from postexposure prophylaxis clinics sponsored by the USPS; 6 workers did not attend the clinics. Of the 94 workers who acquired prophylaxis, only 68 started the antibiotics to prevent anthrax; therefore, of those surveyed, 32 postal workers did not initiate prophylaxis. Postal workers were given ciprofloxacin at initial prophylaxis clinics unless they reported contraindications. Of the 68 postal workers starting antibiotics, 54 persons started ciprofloxacin, 12 doxycycline, and 2 other antibiotics.

Characteristics of the persons who started prophylaxis versus those who did not are presented in Table 1. Male postal workers were 1.5 times more likely to start prophylaxis than female postal workers (relative risk [RR] 1.52; 95% confidence interval [CI] 1.1 to 2.2; $p<0.01$). Persons who reported obtaining an influenza vaccine were more likely to start postexposure prophylaxis (RR 1.26; 95% CI 1.0 to 1.6; $p=0.07$), although this observation did not reach statistical significance. Working on high-speed sorting machines, race, and age were not predictors of starting prophylaxis.

We asked the 32 postal workers who never started postexposure prophylaxis to identify all reasons for declining prophylaxis (Table 2) and to indicate the single most important reason. Nineteen (59%) workers stated that they did not feel they were at personal risk for anthrax. Equal proportions of postal workers (47%) cited negative nasal swabs of workers and concerns about side effects as reasons for not starting prophylaxis. Additional reasons included apprehension about antibiotic resistance, waiting to see if personal exposure had occurred, initial negative environmental samples, and fears that prophylaxis would weaken immune systems. When postal workers were asked to identify the single most important reason for not starting postexposure prophylaxis, 25% of workers reported not personally believing they were at risk for anthrax. An additional 13% cited concerns about side effects as the most important reason for not starting the regimen.

Adherence to Postexposure Prophylaxis

Adherence to the prophylaxis regimen was examined in the 68 workers who started the prophylaxis. We grouped adherence by an average of how many days the worker reported being able to take antibiotic exactly as prescribed. Thirty-one (46%) postal workers reported taking the prophylaxis regimen correctly every day; 23 (34%) took antibiotics correctly 5–6 days per week; and 10 (15%) of workers took antibiotics correctly <4 days per week. Adherence information was not available for four postal workers. Of those starting postexposure prophylaxis, 37 (54%) persons reported missing doses. The top two reasons workers cited for missing a dose were forgetting to take the antibiotic (32%) and side effects (15%).

Table 1. Characteristics of postal workers starting postexposure prophylaxis, Connecticut, 2001

Characteristic	No. of postal workers (n=100)	Relative risk	Confidence interval	p value
Male	63	1.52	1.1 to 2.2	0.004
Influenza vaccine	42	1.26	1.0 to 1.6	0.07
High-speed machine	15	0.86	0.6 to 1.3	0.47
African-American	22	0.78	0.5 to 1.1	0.13
Age <37 years	22	0.98	0.7 to 1.4	0.92
Age ≥52 years	26	0.93	0.7 to 1.3	0.63

Reasons for Stopping Postexposure Prophylaxis

Twenty-one (31%) of 68 postal workers had discontinued the prophylaxis regimen at the time of the survey. We asked these workers to identify all reasons for discontinuation (Table 3) and to indicate the single most important reason why they stopped. Over half (52%) of all who discontinued believed they were not at personal risk or did not believe they had been exposed to *B. anthracis.* Nine (43%) cited side effects as a reason for stopping. Additional concerns were the initial negative environmental findings and the negative nasal swabs. When postal workers were asked to identify the single most important reason for discontinuing prophylaxis, 33% of postal workers reported experiencing side effects; 19% cited initial negative environmental samples from the facility; and 19% did not feel personally exposed.

Side Effects

After susceptibility testing of isolates was confirmed, postal workers were switched to doxycycline by USPS physicians, unless that switch was contraindicated; of 47 workers continuing antibiotics, 43 (91%) were switched to doxycycline during the second round of prophylaxis clinics. Six (13%) workers were switched because of side effects. At the time of the survey, postal workers had taken each medication for approximately the same number of days.

Equal numbers of postal workers surveyed took at least some ciprofloxacin (n=55) and some doxycycline (n=56). Twenty-three (42%) postal workers experienced side effects while taking ciprofloxacin, with 22% reporting multiple symptoms. Twenty-one (38%) postal workers experienced side effects while taking doxycycline, with 21% reporting multiple symptoms. Overall, 35 (51%) of those who began postexposure prophylaxis experienced symptoms while on antibiotics.

Of side effects most frequently reported by postal workers for both antibiotics, the most common were gastrointestinal complaints (Table 4). Diarrhea and abdominal pain were reported by 22% of workers on ciprofloxacin and 13% of workers on doxycycline. Nausea and vomiting were reported by 15% of the postal workers taking ciprofloxacin and 18% taking doxycycline. Fatigue was cited by 9% of the postal workers taking either drug. No significant differences between the proportions of postal workers reporting side effects while taking either medication were reported. No serious side effects were noted.

Only four persons missed work secondary to side effects of the prophylaxis (mean=1 day); only two physician visits for side effects occurred. No hospitalizations were reported.

Discussion

The findings of this study extend the data on adherence with postexposure prophylaxis and substantiate other similar surveys (11). Despite concerns about the safety of postal workers with potential exposures to *B. anthracis*, our survey demonstrates that many workers did not take adequate prophylaxis. Adherence in this population was apparently affected by a low perceived risk for anthrax and a concern about side effects. Concern about side effects was present even before postal workers started taking antibiotics; 47% of the 32 workers who never started prophylaxis cited concern about side effects as a reason. Although many workers did experience side effects, the side effects they reported were not severe. In addition, many postal workers had difficulty taking their medications as prescribed, and they missed doses of prophylaxis.

Two factors may have contributed to the low perceived risk of inhalational anthrax among postal workers. First, results from the first three efforts to collect samples at the postal facilities and the nasal swabs taken at the onset of the investigation were negative for anthrax spores. Second, postal, medical, and union leaders providing information on environmental sampling results and their interpretation at USPS town meetings tried to put the risk in the perspective as explained to them by the Department of Public Health. Overall, the data suggested a possible, but not high, risk for inhalational anthrax. Spores were likely introduced in mid-October before the New Jersey and Washington D.C. regional distribution centers that handled the contaminated Daschle and Leahy letters closed down. Use of compressed air to clean sorting machines, which might have caused aerosolization of spores, had ceased by October 23, when a general USPS advisory against it was circulated. Maximum risk of exposure to aerosolized spores likely occurred during that time. By the time the postexposure prophylaxis clinics began, 30–40 days had passed since the maximum risk period without the occurrence

Table 2. Reasons for postal workers to decline postexposure prophylaxis regimen, Connecticut, 2001

Response	No. of postal workers (n=32)	%
Not at risk for anthrax	19	59
Concerned about side effects from the antibiotics	15	47
Nasal swabs were negative	15	47
Concerned about antibiotic resistance	14	44
Waiting to see if exposed	12	38
Negative environmental samples	12	38
Concerned about weakening immune system	10	31

Table 3. Reasons for discontinuing postexposure prophylaxis regimen, Connecticut, 2001

Response	No. of postal workers (n=21)	%
Not at risk for anthrax	11	52
Not exposed	11	52
Had side effects from the antibiotic	9	43
Nasal swabs were negative	7	33
Negative environmental samples	6	29

Table 4. Side effects reported, by antibiotic, Connecticut, 2001

Side effect reported	Ciprofloxacin (%) (n=55)	Doxycycline (%) (n=56)
Diarrhea/abdominal pain	12 (22)	7 (13)
Nausea/vomiting	8 (15)	10 (18)
Fatigue	5 (9)	5 (9)
Headache	4 (7)	4 (7)
Dizziness	3 (5)	1 (2)
Itching	1 (2)	3 (5)

of any cases of inhalational anthrax in regional facility workers. In addition, the initial samples taken on November 11 and 21, with methods that readily identified spores in New Jersey and Washington, D.C., had failed to identify any spores. These factors were discussed during town meetings in an effort to reassure postal workers, while still emphasizing that a period did occur when spores were in the air, especially around the sorting machines.

In this setting, the numbers of postal workers who accepted antibiotics could not be used as a measure for the numbers of postal workers who actually took prophylaxis. Anecdotally, many postal workers reported obtaining the antibiotics to "have on hand" in the event "I start to feel sick." The postexposure prophylaxis survey was critical in determining the level of adherence and identifying issues affecting adherence in this population.

The circumstances of this prophylaxis campaign, along with the small sample size and potential for recall bias associated with this survey, limit the inferences that may be drawn. For example, some misclassification of side effects as doxycycline- or ciprofloxacin-related may have accompanied the switch in medications. In addition, the study size limits any speculation on reasons why our study found an association between men and starting prophylaxis. Larger postexposure prophylaxis surveys may identify the reason for this and other associations that were not significant in our analysis. Nonetheless, the survey provided important information on adherence to prophylaxis and reasons for nonadherence.

In the event of another bioterrorism attack, public health officials must communicate, early and effectively, the need for potentially exposed persons to initiate and continue postexposure prophylaxis. Specifically, officials should clearly communicate to at-risk persons the explanation that epidemiologic tools such as nasal swabs are poor indicators of past personal exposure and are, at best, indicators only of recent exposure. While important, reassurance must be balanced with clear explanations of risk. Of note in our study is the fact that the one group deemed to be at higher risk—those working on high-speed mail sorting machines—was found no more likely to begin or continue on prophylaxis than persons working elsewhere in the facility.

Potentially exposed persons need to be aware that side effects are to be expected, but that the vast majority of side effects will be mild. Education should center on how to recognize and minimize minor side effects while describing which side effects require immediate medical assistance. Amelioration of side effects is essential if persons are to stay on their regimens, especially if the time period is lengthy. In addition, antibiotic reminder programs such as signs in common areas or buddy systems may improve adherence to postexposure prophylaxis.

In conclusion, if public health officials deem initiating prophylaxis programs necessary, conducting frequent follow-up surveys to measure adherence and identify obstacles to prophylaxis in a specific population will be important in identifying perception problems and maximizing the benefits of preventive therapy.

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References

1. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related inhalational anthrax—Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001;50;1049–51.
2. Barkat LA, Quentzel HL, Jernigan JA, Kirchke DL, Griffith K, Spear SM, et al. Fatal inhalational anthrax in a 94-year old Connecticut woman. JAMA 2002;287:863–8.
3. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax—Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001;50;1077–9.
4. Centers for Disease Control and Prevention. Update: adverse events associated with anthrax prophylaxis among postal employees—New Jersey, New York City, and the District of Columbia metropolitan area, 2001. MMWR Morb Mortal Wkly Rep 2001;50:1051–4.
5. Friedlander AM, Welkos SL, Pitt MLM, Ezzell JW, Warsham PL, Rose KJ, et al. Postexposure prophylaxis against experimental inhalation anthrax. J Infect Dis 1993;167:1239–42.

[1]Greg Armstrong, Nancy Barrett, Kenneth Bell, Mike Bowen, Dave Brownell, Joe Burkhart, Greg Burr, Matt Cartter, Nicole Coffin, Richard Collins, Larry Cseh, Scott Deitchman, Timothy Dignam, Diana Eaton, Devon Eddy, Rick Ehrenberg, Starr Ertel, Brenda Esponda, Marc Fischer, Collette Fitzgerald, Zack Fraser, Julie Gerberding, Mike Grout, Alex Hoffmaster, James Hughes, John Jernigan, Katherine Kelley, Max Kiefer, Bradley King, David Kirschke, Leslye LaClaire, Neil Lustig, Eric E. Mast, Jennifer McClellan, Paul Mead, Richard Meyer, Jasmine Mohammed, Patricia Mshar, Myrth Myers, Randy Nelson, Bruce Newton, Otilio Oyervides, John Painter, Christopher Paddock, Umesh Parashar, Mita Patel, Quyen Phan, Conrad Quinn, Ron Sanders, Susan Smolenski, Karen Spargo, Adrian Stoica, David Sylvain, Fred Tenover, Eyasu Teshale, Rob Weyant, Lynn Wilcox, Alcia Williams, Scott Wright, Ronald Zabrocki, and Sherif Zaki.

6. Cieslak TJ, Eitzen EM. Clinical and epidemiologic principles of anthrax. Emerg Infect Dis 1999;5:552–5.
7. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50;889–93.
8. Centers for Disease Control and Prevention. Notice to readers: interim guidelines for investigation of and response to *Bacillus anthracis* exposures. MMWR Morb Mortal Wkly Rep 2001;50;987–90.
9. Teshale EH, Painter J, Burr GA, Mead P, Wright SV, Cseh LF, et al. Environmental sampling for spores of Bacillus anthracis. Emerg Infect Dis 2002;8;1083-7.
10. Code of Federal Regulations 21CFR 600.80. Updated 2001.
11. Shepard C, the National Anthrax Bioterrorism Response Team. Adverse events associated with postexposure antimicrobial prophylaxis for prevention of anthrax—Florida, New Jersey, New York City, Maryland, Virginia, and Washington, D.C., 2001. Poster session presented at the annual meeting of the Epidemic Intelligence Service, Atlanta, GA, 2002.

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Adherence to Antimicrobial Inhalational Anthrax Prophylaxis among Postal Workers, Washington, D.C., 2001

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In October 2001, two envelopes containing *Bacillus anthracis* spores were processed at the Washington, D.C., Processing and Distribution Center of the U.S. Postal Service; inhalational anthrax developed in four workers at this facility. More than 2,000 workers were advised to complete 60 days of postexposure prophylaxis to prevent inhalational anthrax. Interventions to promote adherence were carried out to support workers, and qualitative information was collected to evaluate our interventions. A quantitative survey was administered to a convenience sample of workers to assess factors influencing adherence. No anthrax infections developed in any workers involved in the interventions or interviews. Of 245 workers, 98 (40%) reported full adherence to prophylaxis, and 45 (18%) had completely discontinued it. Anxiety and experiencing adverse effects to prophylaxis, as well as being <45 years old were risk factors for discontinuing prophylaxis. Interventions, especially frequent visits by public health staff, proved effective in supporting adherence.

In October 2001, two letters with *Bacillus anthracis* spores were mailed to offices on Capitol Hill, Washington, D.C. Both letters were processed at the Washington, D.C., Processing and Distribution Center (DCPDC) of the U.S. Postal Service (USPS). Inhalational anthrax developed in four DCPDC postal workers; two died. More than 2,000 workers and business visitors to the private work areas of DCPDC were potentially exposed to aerosolized *B. anthracis* spores during October 12–21 (1,2). To prevent inhalational anthrax, 60 days of antimicrobial therapy was recommended (primary: ciprofloxacin 500 mg/orally twice a day or doxycycline 100 mg/orally twice a day; alternative: amoxicillin 500 mg/orally twice a day).

Although inhalational anthrax most often develops in the first 7–10 days after exposure, incubation periods as long as 43 days have been reported in Sverdlovsk, Russia (3); in animal studies, inhalational anthrax occurred after 58 days despite 30 days of antimicrobial therapy (4). Therefore, completion of the full 60 days of prophylactic antimicrobial therapy was essential for all postal workers potentially exposed to *B. anthracis* spores at the DCPDC.

Adherence to long-term drug regimens is problematic, and multiple factors influence adherence status, such as regimen factors (e.g., number of pills needed daily), structural factors (e.g., ability to access drugs), individual factors (e.g., cognitive limitations, depression), and health-care provider factors (e.g., ability to listen to and communicate effectively with patients) (5–10). Among the DCPDC workers, typical adherence issues associated with short-course antimicrobial therapy were complicated by the high levels of stress associated with the bioterrorism event and the illnesses and deaths of coworkers, stigma from other postal workers and community members because of erroneous concerns that DCPDC workers were contagious, and the relatively longer duration and potential adverse effects associated with the therapy. The DCPDC facility was closed October 21, 2001, and employees were displaced to work in other area mail facilities, contributing to ongoing disruptions of the workers' daily lives and further complicating adherence. Last, the dynamic nature of the bioterrorist event created a system of evolving health-risk communication that, combined with the many inconsistent sources of information about the event and anthrax, contributed to confusion and misinformation.

In response to the first bioterrorism-related outbreak of inhalational anthrax in the United States, strategies to promote

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adherence to antimicrobial prophylaxis among more than 2,000 DCPDC workers were rapidly implemented. To facilitate future adherence activities in similar events, we evaluated the interventions that were used to support adherence and examined the factors that influenced adherence to the prophylactic regimen in DCPDC workers.

Methods

Qualitative Data Collection

Qualitative data were collected from open-ended interviews (i.e., ones in which interviewer writes down exact responses of interviewee) with convenience samples of the postal worker population throughout the 60-day period to develop and evaluate the interventions and to collect information on the determinants of adherence. The findings from the qualitative interviews were used to develop and validate the close-ended questions (i.e., those with a defined set of answers to choose from, such as yes or no) included in the quantitative survey questionnaire. Information was collected through observation, one-on-one contact, informal small group discussions, and focus group interviews with workers, as well as through interactions with USPS management, worker union representatives, and USPS Employee Assistance Program personnel.

Two staff members from the Centers for Disease Control and Prevention (CDC) conducted five focus group interviews with DCPDC workers during December 13–16, 2001. DCPDC shift supervisors selected six to eight workers to participate in each focus group. During the interviews, workers' responses were noted verbatim on a large flip chart visible to participants at all times. The first author also carried out individual qualitative open-ended interviews during routine interactions with workers throughout December 2001.

The first author conducted all analyses. Notes were immediately reviewed for accuracy at the completion of all interviews and entered into a word-processing software program. Qualitative analysis included several rounds of coding by subject or theme, as well as content analysis and comparison of responses across groups. Analysis focused on both commonly repeated themes (reported by at least 50% of the respondents) and rare points of view.

Interventions to Promote Adherence

To develop appropriate adherence interventions, we obtained support from the USPS management, Employee Assistance Program, and postal service unions. We conducted open-ended interviews with postal workers from various jobs and shifts and incorporated known adherence strategies (5,6,8,10,11) to develop interventions.

Public health staff carried out repeated group question-and-answer sessions and informal contact with workers. These sessions consisted of large and small group and one-on-one interactions to counsel workers. Motivational messages were distributed through the USPS communication infrastructure. In addition, several types of written materials were distributed at the worksite and to workers' homes, including booklets of frequently asked questions about anthrax and antimicrobial therapy, antimicrobial pocket guides with calendar memory aids, and handouts describing ways to minimize stress and recognize the known adverse effects of antimicrobial therapy, such as gastrointestinal upset and yeast infection. Posters and table tents, both with motivational messages, were placed in the workplace. We also provided a letter for workers to take to their personal health-care provider clarifying which area postal workers needed extended prophylaxis and the recommended regimens. This letter was also distributed directly to area health-care providers. Further, after free antimicrobial agents were no longer available, access to antimicrobial agents and reimbursements was facilitated. Finally, clinical team members and a local health-care provider answered specific questions about adverse effects or potential drug interactions, and the local health-care provider consulted with workers free of charge.

In addition, multiple Morbidity and Mortality Weekly Reports (12–14), Health Alert Network alerts, and live broadcasts were disseminated throughout the prophylaxis period to give health-care providers detailed information on which groups needed extended prophylaxis, the recommended regimens, and clinical signs of inhalational anthrax disease.

Quantitative Survey

At five mail facilities, trained interviewers administered a close-ended questionnaire to a convenience sample of all DCPDC employees working the day shift (7 a.m.–3 p.m.) on December 18–20, 2001, days 57–60 of the 60-day regimen. Prophylaxis was first offered October 21, 2001, and most workers picked up prophylaxis on October 22 or 23, 2001. Most (80%) of the displaced DCPDC employees worked at these five facilities. Compared with the day shift, more employees work the swing shift and night shift, when the mail collected during the day is processed.

The questionnaire collected information on demographic characteristics, adherence behaviors, enablers and obstacles to adherence, and information about the implementation of interventions. To assess adherence, workers were asked to respond to five questions located throughout the survey. (For example, "Are you still taking antibiotics for anthrax?" [Possible responses: No, Yes, Declined] and "If you forgot to take any of your pills yesterday, how many pills did you miss?" [Possible responses: None, One, Two, Three.).

Because we were interested in adherence to the recommendation to complete 60 days of prophylaxis, workers were divided into one of three categories. Adherence was defined as full if workers reported they continuously took their antimicrobial therapy throughout the 60-day period, never reduced their dosage, and did not forget any pills the previous day. Adherence was defined as intermediate if workers reduced the dosage, forgot a pill the previous day, or stopped their antimicrobial therapy and restarted at least once. Adherence

was defined as discontinued if workers stopped their antimicrobial therapy and never restarted.

To analyze predictors of nonadherence, we carried out a three-step logistic regression modeling procedure. First, we modeled overall nonadherence (intermediate adherence and discontinued groups combined) compared with full adherence. For this model, we were interested in understanding the differences between those workers who were fully adherent and those who were not fully adherent, including workers who completely discontinued therapy. Second, we modeled intermediate adherence compared with full adherence. For this model, we were interested in understanding the differences between those who were nonadherent but who had not completely discontinued therapy and those who were fully adherent. Third, we modeled the discontinued group compared with the full adherence group. For this model, we were interested in assessing the differences between those who had completely discontinued therapy and those who were fully adherent.

Variables examined were based on previously published articles on adherence and those associated with perceived risk and potential exposure to *B. anthracis* spores in this setting. Inhalational anthrax developed in employees who worked on a sorter machine and in the government mail section of the DCPDC (2). Variables included age, sex, race, perceived risk of breathing in *B. anthracis* spores, work location during exposure period, work description during the time of interview, trouble remembering to take pills, experiencing anxiety, physical signs of stress, severity of adverse effects, and adverse effects negatively affecting work performance. For all analysis, SAS 8.2 (SAS Institute, Inc., Cary, NC) was used. For univariate analysis, two-tailed p values were calculated by chi-square test for dichotomous variables. Potential covariates for the logistic regression models included those with $p<0.20$ in univariate analysis, and possible confounders. We followed a backward elimination strategy to remove nonsignificant covariates in building final parsimonious models. A $p<0.05$ was determined to be statistically significant.

For all qualitative and quantitative interviews, workers were informed that their participation was voluntary and anonymous. Anthrax infections did not develop in any of the workers who participated in the interventions or interviews.

Results

Characteristics of Participants

Of 251 DCPDC workers invited to participate in the questionnaire, 245 (98%) agreed. Among participants, 124 (51%) were male, and 214 (88%) identified themselves as black. Only 1 (0.5%) worker was 18–24 years of age, 74 (30%) were 25–44 years, 163 (67%) were 45–64 years, and 6 (2%) were ≥65 years of age.

Comparison of Adherence among Workers

Among those who completed the questionnaire, 98 (40%) reported full adherence, 45 (18%) discontinued prophylaxis and never restarted, and 102 (42%) were classified as intermediate. Overall, 186 (76%) workers were taking prophylaxis at the time of the interview, including 88 (86%) of the 102 classified as in the intermediate group. Among the intermediate group, 14 (14%) reported discontinuing prophylaxis and restarting at least once, but they were not taking antibiotics at the time of the interview. A total of 45 workers from the discontinued group and 48 workers from the intermediate group reported stopping prophylaxis.

Among the 102 workers classified as intermediate, 40 (39%) reported ever reducing the dosage, 65 (64%) forgot to take at least one pill the previous day, and 48 (47%) reported discontinuing prophylaxis and restarting at least once. Among those who restarted, 20 (42%) missed at least one pill the previous day, and 22 (46%) reported they had ever reduced the dosage.

We examined reasons for stopping prophylactic antimicrobial therapy (Table 1). Most workers reported that several factors influenced their decision to discontinue prophylaxis; 60% cited five or more reasons. Trouble managing adverse effects to antimicrobial agents was the most common reason. Concern over possible long-term adverse effects associated with prolonged antimicrobial therapy was the second most common

Table 1. Reasons for stopping prophylaxis or reducing dosage during anthrax outbreak, Washington, D.C., 2001

Reasons for stopping prophylaxis (n=93)[a]	**n (%)**
Adverse effects	73 (78)
Potential long-term adverse effects	59 (63)
Low risk of developing anthrax disease	47 (51)
Concerns about antibiotic resistance	32 (34)
Negative environmental test results (facility or nasal)	28 (30)
Saving antibiotic for later use	25 (27)
Restrictions to diet or alcohol consumption	22 (24)
Lack of support at work	16 (17)
Difficulty getting appointment with health-care provider	9 (10)
Advised by health-care provider	7 (7)
Expense of health-care provider visit or antibiotic	6 (6)
Reasons for reducing dosage (n=53)[b]	
Adverse effects	38 (72)
Potential long-term adverse effects	8 (15)
Advised by health-care provider	2 (4)
Difficulty remembering to take antibiotic	2 (4)
Take only on workdays	2 (4)
Low supply of pills	1 (2)

[a]Workers were asked to respond to each reason. A total of 45 workers from the discontinued group and 48 workers from the intermediate group reported stopping prophylaxis.
[b]Workers chose only answers that applied. A total of 13 workers from the discontinued group and 40 workers from the intermediate group reported reducing the dosage. Among the 53 workers who reduced their dosage, 5 reported more than one reason, and 5 reported other reasons not included here.

reason for stopping. Similar reasons were given by the workers who reported reducing the dosage of the prescribed antimicrobial therapy. Workers who stopped therapy also reported lacking sufficient information about anthrax and antimicrobial therapy, specifically, information from USPS or CDC.

Predictors of Nonadherence

We wanted to understand the differences between those who were not fully adherent, excluding those who completely discontinued therapy, compared with those who were fully adherent. We therefore modeled intermediate adherence compared with full adherence. Characteristics of these populations and univariate analysis are in Table 2. Independent predictors of intermediate adherence included experiencing "a lot" of adverse effects to antimicrobial therapy, trouble remembering to take pills, as well as age <45 years (Table 3). Experiencing "a lot" of adverse effects, trouble remembering to take pills, and age <45 years were also risk factors for nonadherence in a model combining the intermediate adherence and discontinued groups compared with full adherence (data not shown).

We wanted to understand the differences between those who completely discontinued therapy and those who were fully adherent. We therefore modeled the discontinued group compared with the full adherence group. Characteristics of these populations and univariate analysis can be found in Table 4. Independent predictors of discontinuing therapy included experiencing "a lot" of adverse effects, anxiety, and age <45 years (Table 5). Those workers who reported a high perceived risk of having breathed in *B. anthracis* spores during October 12–21, 2001, were significantly less likely to have discontinued therapy. Those who experienced five or more physical signs of stress were also significantly less likely to have discontinued therapy.

Postal Workers' Experiences and Qualitative Evaluation of Interventions

A total of 38 workers participated in five focus groups, and 22 participated in individual qualitative interviews. The age, sex, and race/ethnic characteristics of qualitative interview participants were similar to those of respondents to the survey questionnaire.

When asked in focus groups and individual qualitative interviews about what adherence interventions were helpful, workers consistently cited repeated visits by public health staff to worksites. Workers reported that the ability to ask personal questions and the distribution of various materials covering multiple health- and work-related issues helped workers complete prophylaxis and promoted adherence by providing accurate and needed information about anthrax, antimicrobial therapy, risk for disease, and the outbreak investigation. Workers reported that this information helped reduce their stress levels and motivated them to continue prophylaxis.

Workers recalled receiving little information at the free antimicrobial distribution sites, and some had forgotten or misunderstood the initial information given. Several opportunities

Table 2. Characteristics of postal workers with intermediate and full adherence to prophylaxis for inhalational anthrax, Washington, D.C., 2001[a]

Characteristics	Intermediate (n=102) n (%)	Full (n=98) n (%)	RR (95% CI)	p value
Sex[b]				
Female	43 (42)	52 (54)	0.8 (0.6, 1.05)	n.s
Male	59 (58)	45 (46)	Ref	-
Age,[b] (y)				
18–44	34 (33)	16 (16)	2.0 (1.2, 3.4)	p<0.05
≥45	68 (67)	81 (84)	Ref	-
Race/ethnicity[b]				
Black	90 (88)	88 (91)	1.0 (0.9, 1.1)	n.s.
Other	5 (5)	3 (3)	1.6 (0.4, 6.5)	n.s.
White	7 (7)	6 (6)	Ref	-
Work description at interview[c]				
Driver	12 (12)	8 (8)	1.4 (0.6, 3.3)	n.s.
Government mail	17 (16)	21 (21)	0.8 (0.4, 1.4)	n.s.
Administration	7 (7)	6 (6)	1.1 (0.4, 3.2)	n.s.
Plant floor	66 (65)	63 (64)	Ref	-
Worked on sorter or in government mail section[d]				
Yes	70 (72)	70 (75)	0.9 (0.8, 1.1)	n.s.
No	27 (28)	23 (25)	Ref	-
Perceived risk[e]				
High	58 (57)	60 (61)	0.9 (0.7, 1.2)	n.s.
Some	39 (38)	35 (36)	1.1 (0.7, 1.5)	n.s.
None	5 (5)	3 (3)	Ref	-
Adverse effects[f]				
A lot	20 (20)	9 (9)	2.1 (1.02, 4.4)	p<0.05
Some	54 (53)	48 (49)	1.1 (0.8, 1.4)	n.s.
Not at all	28 (27)	41 (42)	Ref	-
Physical signs of stress[g]				
5–11 signs	37 (36)	28 (29)	1.3 (0.8, 1.9)	n.s.
1–4 signs	50 (49)	57 (58)	0.8 (0.6, 1.1)	n.s.
0 signs	15 (15)	13 (13)	Ref	-
Anxiety[h]				
Yes	37 (36)	33 (34)	1.1 (0.7, 1.6)	n.s.
No	65 (64)	65 (66)	Ref	-
Trouble remembering pills[i]				
Yes	67 (66)	44 (45)	1.5 (1.1, 1.9)	p<0.05
No	35 (34)	54 (55)	Ref	-
Worse work performance[j]				
Yes	16 (16)	15 (15)	1.0 (0.5, 1.9)	n.s.
No	86 (84)	83 (85)	Ref	-

[a]RR, relative risk; 95% CI, 95% confidence interval; n.s., not statistically significant; ref, referent.
[b]One missing value for full adherence.
[c]Work location during the survey interview, December 18–20, 2001.
[d]Worked close to these areas for more than half of the normal workdays during exposure period of October 12–21, 2001. Responses of "don't know" were excluded from analysis (n=13).
[e]Perceived risk of breathing in *Bacillus anthracis* spores during exposure period of October 12–21, 2001.
[f]Reported how much side effects affected their lives.
[g]Physical signs of stress included fatigue, headaches, chest pain, rapid heartbeat, unplanned changes in weight, less sleep or difficulty sleeping, muscle tremors or twitches, difficulty or rapidity in breathing, elevated blood pressure, nausea or vomiting, and dizziness or lightheadedness.
[h]Reported they experienced anxiety since anthrax events started. Anxiety was one of 22 listed physical, emotional, mental, and behavioral signs of stress on our questionnaire.
[i]Reported they sometimes or almost always had trouble remembering their pills.
[j]Reported side effects negatively affected their work performance.

Table 3. Predictors of intermediate adherence (n=102) compared with full adherence (n=98), Washington, D.C., 2001[a]

Predictor covariates		Adjusted, OR (95% CI)	p value
Age	18–44 y	2.2 (1.1, 4.4)	p<0.05
Adverse effects[b]	A lot	2.8 (1.1, 7.4)	p<0.05
	Some	1.5 (0.8, 2.8)	n.s.
	Not at all	ref	-
Trouble remembering pills[c]	Yes	2.2 (1.2, 4.1)	p<0.05

[a] OR, odds ratio; 95% CI, 95% confidence interval; n.s., not statistically significant; ref, referent.
[b]Reported how much side effects affected their life.
[c]Reported they sometimes or almost always had trouble remembering their pills.

to speak with public health staff were necessary to clarify questions, especially as new issues arose. However, some workers complained that public health staff could not provide adequate answers to all their questions, such as those related to the long-term status of viable *B. anthracis* spores inhaled into the lung, the long-term effects of extended antimicrobial therapy, environmental sampling results, the need for personal protective gear, and other occupational health concerns.

In the questionnaire, 82% of workers reported they wanted to receive public health information in a variety of formats, including both orally and written, as well as information from the media. The questionnaire showed that only 3% of workers did not participate in oral communication interventions, 2% did not receive written materials distributed to employees at the worksite or at their homes, and 21% did not see posted signs and messages at work.

Discussion

After the first bioterrorism-related anthrax outbreak in the United States, we rapidly developed and implemented multiple adherence interventions to prevent inhalational anthrax in >2,000 DCPDC workers. This was the first time adherence interventions have been conducted and evaluated in an applied public health bioterrorism response. Our interventions promoted the message that adherence was essential for the full 60 days of antimicrobial therapy. Further, the interventions were carried out during the entire 60-day period. Seventy-six percent of postal workers were taking antimicrobial prophylaxis at the time of the evaluation. Despite differences in assessing adherence, the adherence found in this study was relatively high compared with other studies of adherence to short-course antimicrobial therapy. For example, Ley (15) reported approximately 50% adherence in a review of adherence studies to short-course antibiotics, and Brookoff (16) reported only 31% adherence to a 10-day course of doxycycline (n=386) for outpatient treatment of pelvic inflammatory disease.

Many issues hindered adherence in this anthrax outbreak, including adverse effects of the antimicrobial prophylaxis, such as gastrointestinal upset and yeast infection, trouble remembering to take the pills, perceived risk, anxiety, and physical signs of stress. Although these factors occurred in the context of a bioterrorism event, similar adherence obstacles

Table 4. Characteristics of postal workers who discontinued or were fully adherent to prophylaxis for anthrax, Washington, D.C., 2001[a]

Characteristics	Discontinued (n=45) n (%)	Full adherence (n=98), n (%)	RR (95% CI)	p value
Sex[b]				
Female	25 (56)	52 (54)	1.0 (0.7, 1.4)	n.s.
Male	20 (44)	45 (46)	Ref	-
Age[b]				
18–44 y	25 (56)	16 (16)	3.4 (2.0, 5.7)	p<0.05
≥45 y	20 (44)	81 (84)	Ref	-
Race/ethnicity[b]				
Black	36 (80)	88 (91)	0.9 (0.7, 1.04)	n.s.
Other	3 (7)	3 (3)	2.2 (0.4, 10.4)	n.s.
White	6 (13)	6 (6)	Ref	-
Work description at interview[c]				
Driver	6 (13)	8 (8)	1.6 (0.6, 4.4)	n.s.
Government mail	3 (7)	21 (21)	0.3 (0.1, 0.99)	p<0.05
Administration	12 (27)	6 (6)	4.3 (1.7, 10.9)	p<0.05
Plant floor	24 (53)	63 (64)	Ref	-
Worked on sorter or in government mail section[d]				
Yes	18 (43)	70 (75)	0.6 (0.4, 0.8)	p<0.05.
No	24 (57)	23 (25)	Ref	-
Perceived risk[e]				
High	16 (35)	60 (61)	0.6 (0.4, 0.9)	p<0.05
Some	25 (56)	35 (36)	1.5 (1.1, 2.2)	p<0.05
None	4 (9)	3 (3)	Ref	-
Adverse effects[f]				
A lot	11 (25)	9 (9)	2.7 (1.2, 6.0)	n.s.
Some	19 (42)	48 (49)	0.9 (0.6, 1.3)	n.s.
Not at all	15 (33)	41 (42)	Ref	-
Physical signs of stress[g]				
5–11 signs	7 (16)	28 (29)	0.5 (0.2, 1.1)	n.s.
1–4 signs	29 (64)	57 (58)	1.1 (0.8, 1.4)	n.s.
0 signs	9 (20)	13 (13)	Ref	-
Anxiety[h]				
Yes	17 (38)	33 (34)	1.1 (0.7, 1.8)	n.s.
No	28 (62)	65 (66)	Ref	-
Trouble remembering pills[i]				
Yes	23 (51)	44 (45)	1.1 (0.8, 1.6)	n.s.
No	22 (49)	54 (55)	Ref	-
Worse work performance[j]				
Yes	9 (20)	15 (15)	1.3 (0.6, 2.7)	n.s.
No	36 (80)	83 (85)	Ref	-

[a]RR, relative risk; 95% CI, 95% confidence interval; n.s., not statistically significant.
[b]One missing value for full adherence.
[c]Work location during the survey interview, December 18–20, 2001.
[d]Worked close to these areas for more than half of the normal workdays during exposure period of October 12–21, 2001. Responses of "don't know" excluded from analysis (n=13).
[e]Perceived risk of breathing in *Bacillus anthracis* spores during exposure period of October 12–21, 2001.
[f]Reported how much side effects affected their lives.
[g]Physical signs of stress included fatigue, headaches, chest pain, rapid heartbeat, unplanned changes in weight, less sleep or difficulty in sleeping, muscle tremors or twitches, difficulty or rapidity in breathing, elevated blood pressure, nausea or vomiting, and dizziness or lightheadedness.
[h]Reported they experienced anxiety since anthrax events started. Anxiety was one of 22 listed physical, emotional, mental, and behavioral signs of stress on our questionnaire.
[i]Reported they sometimes or almost always had trouble remembering their pills.
[j]Reported side effects negatively affected their work performance.

Table 5. Predictors of discontinued therapy (n=45) compared with full adherence (n=98), Washington, D.C., 2001[a]

Predictor covariates		Adjusted, OR (95% CI)	p value
Age	18-44 y	6.7 (2.6, 17.3)	p<0.05
Perceived risk[b]	High	0.1 (0.01, 0.8)	p<0.05
	Some	0.4 (0.1, 3.0)	n.s.
	None	ref	-
Adverse effects[c]	A lot	20.4 (3.0, 140.1)	p<0.05
	Some	1.7 (0.6, 5.1)	n.s.
	Not at all	ref	-
Physical signs of stress[d]	5-11 signs	0.02 (0.003, 0.2)	p<0.05
	1-4 signs	0.3 (0.1, 1.1)	n.s.
	0 signs	Ref	-
Anxiety[e]	Yes	3.5 (1.1, 10.9)	p<0.05

[a]OR, odds ratio; 95% CI, 95% confidence interval; n.s., not statistically significant; ref, referent.
[b]Perceived risk of breathing in *B. anthracis* spores during exposure period October 12–21, 2001.
[c]Reported how much side effects affected their life.
[d]Physical signs of stress included fatigue, headaches, chest pain, rapid heartbeat, unplanned changes in weight, less or difficulty sleeping, muscle tremors or twitches, difficulty or rapid breathing, elevated blood pressure, nausea or vomiting, and dizziness or lightheadedness.
[e]Reported they experienced anxiety since anthrax events started. Anxiety was one of 22 listed symptoms of stress on our questionnaire.

have been reported elsewhere (5,7,17,18). Additional issues complicating adherence among postal workers included the large number of workers affected, occupational health and other work-related issues, limited capacity of local departments of health to undertake a program to promote adherence for a large number of people in an emergency, and the hysteria and media coverage associated with this bioterrorism event, which likely magnified miscommunication and workers' confusion.

In developing the intervention protocols, we drew upon lessons learned from adherence strategies for isoniazid treatment for latent tuberculosis infection and highly active antiretroviral therapy for HIV infection. Studies of these strategies conclude that interventions must be multifaceted, ongoing, flexible, individualized, and repetitive to achieve optimal adherence levels (5,8,9,18–20). Our interventions included many of these characteristics, such as repeated visits, clarifying questions, counseling workers, incorporating pill-taking into daily routines, and providing workers with as much information as possible about anthrax and antimicrobial therapy. Inhalational anthrax as a disease and bioterrorism-associated disease are complex issues and relaying this information to people was difficult. Therefore, multiple formats (verbal, written, and graphic) were necessary to effectively communicate information to workers.

Many workers mistook signs of stress (e.g., complaints of fatigue, lack of sexual drive, and increased crying) for adverse effects of the antimicrobial therapy. Further, the stress associated with the bioterrorist event magnified the adverse effects associated with prophylaxis. For some symptoms, distinguishing between adverse effects of stress and those of the antimicrobial therapy, such as gastrointestinal upset, was impossible. Those who worked close to areas where coworkers with inhalational anthrax had worked reported more physical signs of stress, had a higher perceived risk of having breathed in *B. anthracis* spores, and were also more likely to have continued therapy. Those who had anxiety were more likely to have discontinued therapy. Published articles report associations between anxiety or depression and nonadherence (7,17), and some researchers posit that the inability to cope with anxiety is the better predictor of nonadherence (17). These findings highlight the importance of communicating early and repeatedly the known adverse effects people should expect, and how to manage all potential effects, including those caused by prophylaxis and stress or anxiety related to bioterrorist events.

Only self-reports were collected to assess adherence in this evaluation. Several studies suggest that self-reporting overestimates adherence, while reports of nonadherence are usually valid (5,7). Therefore, our results may have overestimated adherence, but it is unlikely that we overestimated the number of persons who discontinued prophylaxis. Data were collected from a convenience sample and may not be representative of all DCPDC workers. A March 2002 phone survey among DCPDC workers (62% response rate) reported similar age, sex, and race/ethnicity characteristics (21). Because we did not have a control group who did not receive interventions to promote adherence, we cannot measure the effectiveness of our interventions; however, our adherence findings were similar to those of other studies that were not implemented in the setting of a bioterrorist emergency response (7,8,11). In addition, the evaluation was conducted during the holiday season, the busiest time of the year for the USPS, and we were permitted to conduct the questionnaire only with workers on the day shift (7 a.m.–3 p.m.). The experiences of day-shift workers may be different from those who work other shifts, although, based on the qualitative findings carried out with workers from all shifts and the continual interactions with workers throughout the 60-day period, these findings likely reflect the experiences of most DCPDC workers. Last, our evaluation may have been affected by the general media coverage of the bioterrorism events.

Nonadherence is common and should be expected in all settings, especially in a bioterrorism-related context that involves further challenges and complications to adherence. Considering the large number of workers who took less than the recommended regimen, evaluating adherence promotion interventions during bioterrorist outbreaks is very important. In emergency settings, adherence programs may overburden local departments of health because they require ongoing personal interactions and are labor-intensive when large numbers of people are affected. Efforts to develop a plan to promote adherence in the event of a bioterrorism outbreak, which could be tailored to the situation and implemented immediately, will aid future public health emergency responses where adherence

to recommended prophylaxis is necessary to save lives. During occupational exposures, supplementing occupational health resources may be necessary. To optimally promote adherence, such plans should incorporate continual interaction with the affected persons, provide consistent and clear messages, and include interventions that help persons incorporate pill-taking into daily routines and manage known adverse effects, including those caused by prophylaxis, anxiety, and stress related to bioterrorism events.

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References

1. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
2. Dewan PK, Fry AM, Laserson K, Tierney BC, Quinn CP, Hayslett JA, et al. Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. Emerg Infect Dis 2002;8;1066-72.
3. Meselson M, Guillemin J, Hugh-Jones M, Langmuir A, Popova I, Shelokov A, et al. The Sverdlovsk anthrax outbreak of 1979. Science 1994;266:1202–8.
4. Friedlander A, Welkos S, Pitt M, Ezzell J, Worsham P, Rose K, et al. Postexposure prophylaxis against experimental inhalation anthrax. J Infect Dis 1993;167:1239–42.
5. Sumartojo E. When tuberculosis treatment fails: a social behavioral account of patient adherence. Am Rev Respir Dis 1993;147:1311–20.
6. Sumartojo E. Adherence to the tuberculosis treatment plan. In: Cohen F, Durham J, editors. Tuberculosis: a sourcebook for nursing practice. New York: Springer Publications; 1995. p. 121–36.
7. Chesney M, Morin M, Sherr L. Adherence to HIV combination therapy. Soc Sci Med 2000;50:1599–605.
8. Chesney M. Factors affecting adherence to antiretroviral therapy. Clin Infect Dis 2000;30:S171–6.
9. Sanson-Fisher R, Bowman J, Armstrong S. Factors affecting nonadherence with antibiotics. Diagn Microbiol Infect Dis 1992;15:103S–9S.
10. Catz S, Kelly J, Bogart L, Benotsch E, McAuliffe T. Patterns, correlates, and barriers to medication adherence among persons prescribed new treatments for HIV disease. Health Psych 2000;19:124–33.
11. Lerner B, Gulick R, Dubler N. Rethinking nonadherence: historical perspectives on triple-drug therapy for HIV disease. Ann Intern Med 1998;129:573–8.
12. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–93.
13. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:909–19.
14. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001;50:941–8.
15. Ley P. Satisfaction, compliance and communication. Br J Clin Pharmacol 1982;21:241–54.
16. Brookoff D. Compliance with doxycycline therapy for outpatient treatment of pelvic inflammatory disease. South Med J 1994;87:1088–91.
17. Dunbar-Jacobs J, Schlenk E, Burke L. Predictors of patient adherence: patient characteristics. In: Shumaker S, Schron E, Ockene J, McBee W, editors. The handbook of health behavior change. 2nd edition. New York: Springer Publishing Co.; 1998. p. 491–511.
18. Tsasis P. Adherence assessment to highly active antiretroviral therapy. AIDS Patient Care and STDs 2001;15:109–15.
19. Stone V. Strategies for optimizing adherence to highly active antiretroviral therapy: lessons from research and clinical practice. Clin Infect Dis 2001;33:865–72.
20. Haynes R, Wang E, Gomes M. A critical review of interventions to improve compliance with prescribed medications. Patient Education and Counseling 1987;10:155–66.
21. Shepard CW, Soriano-Gabarro M, Zell ER, Hayslett J, Lukacs S, Goldstein S, et al. Antimicrobial postexposure prophylaxis for anthrax: adverse events and adherence. Emerg Infect Dis 2002;8;1124-32.

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Surface Sampling Methods for *Bacillus anthracis* Spore Contamination

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During an investigation conducted December 17–20, 2001, we collected environmental samples from a U.S. postal facility in Washington, D.C., known to be extensively contaminated with *Bacillus anthracis* spores. Because methods for collecting and analyzing *B. anthracis* spores have not yet been validated, our objective was to compare the relative effectiveness of sampling methods used for collecting spores from contaminated surfaces. Comparison of wipe, wet and dry swab, and HEPA vacuum sock samples on nonporous surfaces indicated good agreement between results with HEPA vacuum and wipe samples. However, results from HEPA vacuum sock and wipe samples agreed poorly with the swab samples. Dry swabs failed to detect spores >75% of the time when they were detected by wipe and HEPA vacuum samples. Wipe samples collected after HEPA vacuum samples and HEPA vacuum samples collected after wipe samples indicated that neither method completely removed spores from the sampled surfaces.

The Brentwood Mail Processing and Distribution Center in Washington, D.C., was extensively contaminated with *Bacillus anthracis* spores after two letters containing spores were processed at this facility on October 12, 2001 (1). Subsequently, inhalational anthrax developed in four postal workers. An investigation in late October 2001, using surface wipe and HEPA vacuum sock sampling techniques, showed widespread *B. anthracis* spore contamination inside the building. Spore concentrations were particularly high around Delivery Bar Code Sorter (DBCS) machine no. 17, which had processed the letters, and in the government mail area, where the letters had been processed before being distributed.

This report describes the results of sampling for *B. anthracis* spores in an investigation conducted December 17–19, 2001, by the Centers for Disease Control and Prevention (CDC), the Agency for Toxic Substances and Disease Registry (ATSDR), the U.S. Postal Service (USPS), and a USPS contractor. At the time of this investigation, technical issues regarding sampling and analyses for *B. anthracis* spores remained unresolved, such as which technique for surface sampling (swabs, wipes, or HEPA vacuum socks) is most appropriate for collecting spores in specific situations, how the different types of surface sampling methods compare, and how effectively the sampling methods collect spores from contaminated surfaces. Surface sampling to determine the presence of *B. anthracis* spores in an environment is essential for determining extent of contamination, assessing potential for exposure and need for medical treatment, and guiding clean-up and reentry efforts.

Sampling methods (swabs, wipes, rinses, direct agar contact, and vacuuming) have been evaluated for collecting microorganisms from surfaces (2–7), primarily in laboratory settings. *B. subtilis* spores, which may behave much like *B. anthracis* spores, have been frequently used as the microbiologic agent sampled. Substantial variation in sample recoveries was observed for the various methods. In addition, the methods have not been validated specifically for collecting and analyzing *B. anthracis* spores in environmental samples. The primary objective of our survey was to compare the levels of *B. anthracis* spores in side-by-side samples obtained by the surface swab, wipe, and HEPA vacuum sock methods to evaluate their relative effectiveness.

USPS representatives and a USPS contractor had conducted clean-up operations at the Brentwood facility since late October. However, much of the facility had not been cleaned and was believed still contaminated with *B. anthracis* spores. Even though the DBCS machine (no. 17) that processed the contaminated letters had been cleaned by HEPA vacuum, washed with a 10% sodium hypochlorite solution followed by neutralization with a sodium thiosulfate solution, and rinsed with water, this machine was reportedly still contaminated with *B. anthracis* spores (8). For these reasons, the Brentwood facility was thought to be a good location to compare surface sampling and analytical methods.

Methods

Surface sampling was conducted by using swabs, wipes, and HEPA vacuum socks. To compare the sampling techniques,

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we selected locations where the three types of samples could be collected adjacent to each other on nonporous surfaces, with an emphasis on locations believed to be still contaminated with *B. anthracis* spores. The locations sampled included the surfaces of selected DBCS machines (particularly machine no. 17), return air ducts, tops of the window boxes along the postal inspector walkways, and the tops of mail sorting bins in a secure area approximately 23 m from DBCS machine no. 17. The order in which the samples were collected varied in a randomized fashion from location to location; each site was assigned a location number and sampled according to a predetermined, randomized plan. We used the randomized sampling plan to reduce sampling biases that might be caused by nonuniform distribution of spores across surfaces and affected by the order in which samples were collected.

Seven swab, six wipe, and five HEPA vacuum sock samples were collected as control samples; that is, these samples were handled in the same way as others but not used to sample any surfaces. The purpose of these control samples was to evaluate the potential contamination of sample media, unrelated to actual sample collection.

Nine additional blank HEPA vacuum sock samples were collected to estimate cross-contamination by inserting them into the vacuum nozzle after a HEPA vacuum sock sample had been collected and the nozzle cleaned; these socks were then withdrawn and placed in a sterile conical tube for laboratory analysis.

Investigators were given written instructions for collecting samples at each selected location (Figure 1). The surface areas sampled by each technique were intended to be comparable, but not necessarily equal. In particularly dirty areas, swabs and wipes could not cover as large a surface area as the HEPA vacuum sock samples without becoming overloaded; investigators were instructed to avoid overloading the samples by reducing the size of the surface sampled.

The following procedures, used to collect the three types of surface samples, were recommended for collecting surface environmental samples for culturing *B. anthracis* (9). The surface samples were all collected after investigators had donned nonpowdered gloves over two pairs of nitrile protective gloves, as part of the personal protective gear. The area of the surface sampled was measured with a tape measure and recorded in square centimeters.

Swab samples were collected by removing a sterile, rayon (noncotton) swab (Environmental Swab Kit, CDC, Atlanta, GA) from a sterile tube, moistening it by inserting it into a second tube which contained a sponge soaked with sterile 1.5 mL of phosphate-buffered saline (PBS) at pH 7.2, and then swabbing the selected surface by moving the swab back and forth across the surface with several horizontal strokes, then several vertical strokes. The swab was rotated during sampling to ensure that the entire surface of the swab was used. After sampling, the swab was returned to its original, prelabeled sampling tube for submission to the laboratory. At every selected location, premoistened swabs were collected. Approximately half the sites were also sampled again with unmoistened dry swabs to compare the sampling efficiency of dry swabs to wet swabs and other techniques.

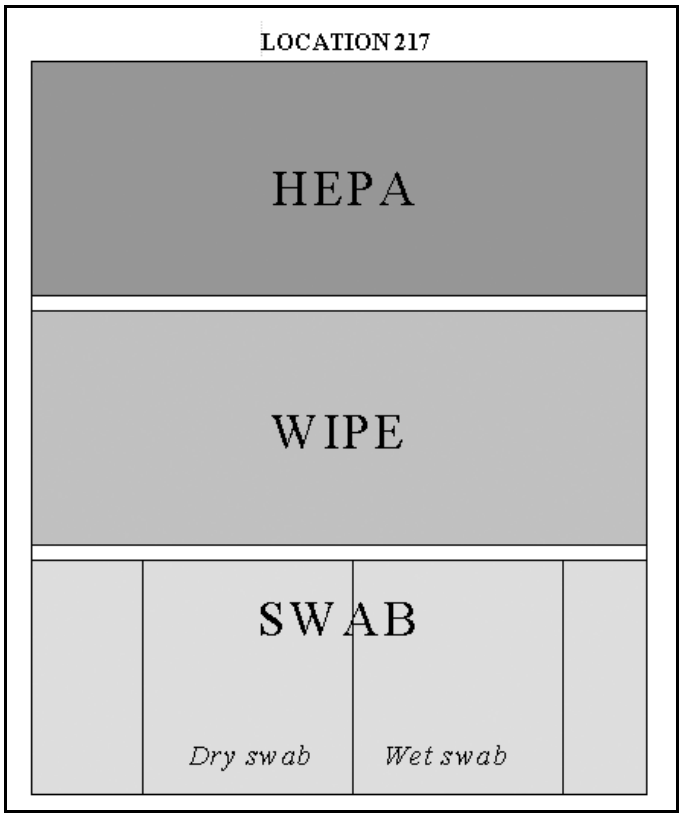

Figure 1. Sample instructions for collection of swab, wipe, and HEPA vacuum sock samples, Brentwood Mail Processing and Distribution Center, 2001. For specific location, investigator was given these instructions (exact text follows). Divide the selected space into three sections where each of the three types of surface samples (swab, wipe, HEPA vacuum sock) may be collected. Follow the random key above to designate which section will be sampled by each method and in which order the samples will be collected (follow top to bottom). Record the area of surface sampled by each method. The surface areas need not be equal, but should be sufficient to provide adequate sample collection for each method. Sample order for location was: 1) Collect the HEPA vacuum sock sample first and record surface area. After sampling, clean vacuum nozzle with alcohol and insert clean vacuum sock; remove this sock without sampling to serve as "contamination blank." 2) Collect the WIPE sample second and record surface area. 3) Collect the SWAB samples third and record surface area. The first swab sample should be collected without moistening it. The second swab sample should be sampled pre-moistened. Take care not to overload swabs. 4) Collect an additional WIPE sample across the entire area which had been sampled by HEPA vacuum sock. 5) Collect an additional HEPA vacuum sock sample across the entire which had been sampled by WIPE.

Wipe samples were collected on selected surfaces with a 7.62 x 7.62 cm sterile rayon gauze pad (Dukal Corp., Syosset, NY) premoistened with approximately 5 mL sterile water (Baxter Healthcare Corp., Deerfield, IL). The surface was thoroughly wiped back and forth by using several vertical strokes, folding the exposed side of the pad, and making several horizontal strokes over the same area with the other side of the wipe. The pad was then placed in a prelabeled, 50-mL sterile conical tube and sealed with a cap.

HEPA vacuum sock samples were collected by inserting a cone-shaped filtering trap (dust collection filter sock; Midwest

Filtration Co., Fairfield, OH) into the nozzle of a HEPA vacuum cleaner (Atrix International Inc., Burnsville, MN). The vacuum had an electric motor (120 V, 6.6 A, 1 hp) to provide suction of 28 cubic feet (792.4 L) per min through the vacuum nozzle (Figure 2). The plastic sleeve of the dust collection trap was folded over the outside of the nozzle and held in place by hand while the vacuum nozzle was moved slowly back and forth across the sampled surface. The dust collection trap was removed from the vacuum nozzle, placed in a prelabeled, 50-mL sterile conical tube, and sealed with a cap. Before inserting a clean sock into the vacuum nozzle and collecting a subsequent sample, the investigator put on a new pair of gloves and wiped the inside of the vacuum nozzle thoroughly with an alcohol wipe, to physically remove contamination from the nozzle surface (not to sterilize the surface because alcohol does not effectively kill *B. anthracis* spores [10]). To determine whether cross-contamination of subsequent vacuum samples might occur through contamination of the vacuum nozzle during sampling, occasionally a filter sock was inserted into the vacuum nozzle after a sample had been collected and the nozzle cleaned, but the sock was then simply withdrawn and placed in a sterile conical tube for laboratory analysis.

Swab and wipe samples were extracted in a laboratory operated by the USPS contractor at the Brentwood facility. The samples were extracted by adding 20–30 mL 0.3% Tween 20 in PBS to a 50-mL Blue Falcon screw-top tube (Becton Dickinson Labware, Franklin Lakes, NJ) and vortexing the tube for 3 min. The contents of the tube were allowed to settle for 5 min, and swabs and wipes were removed. The tube was centrifuged at 3,000–4,500 rpm, 15–30 min at 10°C, the supernatant removed by decanting, and the pellet was resuspended in 2 mL 0.3% Tween 20 in PBS solution. Approximately half the resuspended extract was shipped to CDC Bioterrorism Surge Capacity and Anthrax Laboratories for culture and confirmatory analysis. The remaining half of the resuspended extract was retained at the laboratory at the Brentwood facility for polymerase chain reaction (PCR) analysis (unpub. data).

At CDC, 0.1 mL of the suspension (approximately 10% of the extract solution) was plated to trypticase soy agar with 5% sheep blood and streaked for quantification. The plates were incubated at 35°C–37°C in ambient air and examined after 24 h and 48 h. Suspect colonies were screened by Level A procedures for identification of *B. anthracis* (11). Identification of all strains was confirmed by standard microbiologic procedures and the Laboratory Response Network (LRN) testing algorithm (12,13). Results of these samples were reported as number of CFUs per plate. To estimate CFUs per sampled surface area, the number of CFUs per plate was multiplied by 20 (2 mL extract solution divided by 0.1 mL plated solution) and divided by the recorded surface area in square centimeters. When the number of colonies on the culture plates exceeded approximately 300, they were reported as too numerous to count.

The HEPA vacuum sock samples were analyzed by an LRN contract laboratory. The HEPA vacuum socks and their contents were weighed on a precision balance. We used the average weight of five unused sock samples to estimate the presampling weight of the vacuum socks; the average weight of the unused socks was 0.70 g (standard deviation 0.02 g). The average weight was subtracted from the postsampling weight of each sock sample to determine the weight of its contents. Approximately 20–30 mL 0.3% Tween 20 in PBS was added to a 50-mL cup containing the sock and its contents and placed on a shaker for 30 min. The contents of the cup were allowed to settle for 5 min; the supernatant then was poured into a 50-mL Blue Falcon screw-top tube (Becton Dickinson Labware). The tube was centrifuged at 3,000–4,500 rpm, for 15–30 min at 10°C; approximately 90% of the starting volume was then removed. The pellet in the bottom of the tube was resuspended in approximately 2 mL 0.3% Tween 20 in PBS, and 0.1 mL (two drops from Pasteur pipette) and 0.01 mL (added by using a calibrated loop) of the suspension were plated to two trypticase soy agar plates with 5% sheep blood and streaked for quantification. The plates were incubated and screened, and suspect colonies were identified by using the same laboratory methods used for the swab and wipe samples. Results of these samples were reported as CFU/g of material collected; the estimated weights of the sock contents were also reported. To estimate CFU per sampled surface area, the reported CFU/g were multiplied by the reported weight of the sock contents and divided by the recorded surface area in square centimeters.

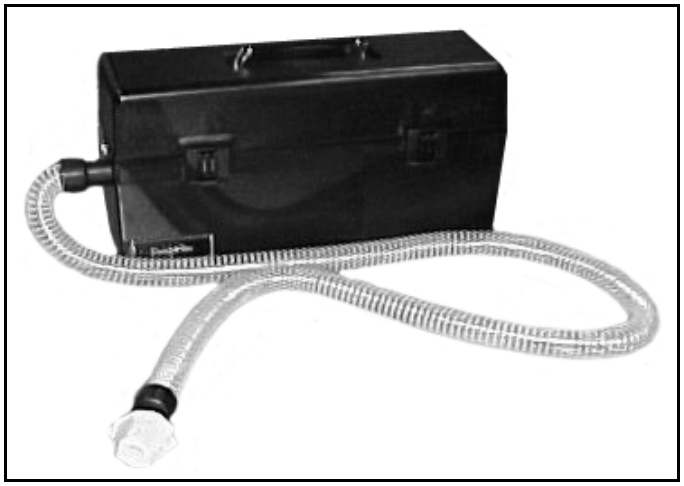

Figure 2. Photograph of HEPA vacuum cleaner and sock sample.

To evaluate the effectiveness of the wipe and HEPA vacuum samples for removing spores from surfaces, at some locations we collected wipe samples over the same surface area previously vacuumed, as well as HEPA vacuum samples over the same surface area previously wiped. We compared the relative difference in CFU/cm^2 reported for the two methods to evaluate the removal efficiency of the wipe and HEPA vacuum sock samples.

Operations to decontaminate the Brentwood facility had been done since late October 2001 by using HEPA vacuums and sodium hypochlorite solutions. These clean-up operations

focused on the DBCS machines. Swab, wipe, and HEPA vacuum sock samples of DBCS machine surfaces that had been cleaned were collected to evaluate the effectiveness of cleanup operations.

PC-SAS computer software was used for all statistical analyses (14). Sample results (positive vs. negative) were analyzed by using simple descriptive statistics, including counts and percents. Agreement between paired sampling methods was assessed by using Cohen's Kappa, a statistical method that measures agreement beyond what would be expected based on chance alone (15). Kappa scores <0.4 were considered poor agreement, while scores >0.75 indicated excellent agreement; Kappa scores between these values indicated fair to good agreement. Sample levels (CFU/cm^2) were analyzed by simple descriptive statistics, including sample median and range. Spearman's rank correlation coefficient significance tests that do not assume normality were used as a measure of the association between two paired sampling methods (16). Agreement between paired sampling methods with respect to ordered categories (0, 0.1–1.6, 1.7–15.5, and >15.5 CFU/cm^2) was assessed by using Kendall's tau-b statistic, which measures ordinal association (17).

Results

Descriptive statistics for the culture analysis of the dry and wet swab, wipe, and HEPA vacuum sock samples are shown in Table 1. *B. anthracis* was cultured from 4 (14%) of 28 dry swab samples, while 36 (54%) of 67 wet swabs were culture positive. Fifty-eight (87%) of 67 of the wipe samples and 51 (80%) of 64 of HEPA vacuum sock samples were culture positive. Although CFUs/cm^2 were reported for each positive sample, these results should only be considered semiquantitative; absolute concentrations cannot be directly compared across the sampling methods. However, the calculated concentrations of *B. anthracis* spores in the culture-positive HEPA vacuum sock samples tended to be greater than in the other types of samples.

None of the blank control samples was positive for *B. anthracis* spores. Of the nine blank HEPA vacuum samples collected from the vacuum nozzle to estimate cross-contamination, eight were culture negative; one *B. anthracis* CFU was detected in one sock.

The results of the dry swab samples are compared with results obtained by using the other types of samples (Table 2). Dry swab samples were collected at 28 locations. These results indicate that when corresponding wipe and HEPA vacuum sock samples were culture positive for *B. anthracis* spores, the dry swab samples detected *B. anthracis* 4 of 23 times. When the corresponding wet swabs were positive for *B. anthracis* spores, the dry swabs detected *B. anthracis* 4 of 13 times. At no time were the dry swabs positive while the other types of corresponding samples did not detect spores. Results of the dry swabs were not included in further comparisons.

A total of 58 sets of wet swab, wipe, and HEPA vacuum sock samples collected side-by-side were available for comparison, and 67 sets of wet swab and wipe samples collected side-by-side were also available for comparison (Table 3). Results of wet swab and wipe sample analysis were concordant in 64% of the sample comparisons; 23 wipe samples were reported as culture positive when the wet swab samples failed to detect spores, and 1 culture-positive wet swab sample was reported when the corresponding wipe sample was culture negative. Results of the wet swab and HEPA vacuum sock samples were also concordant on 64% of the sample comparisons with similar results as the wet swab and wipe comparison. Twenty-one (36%) HEPA vacuum samples were reported as culture positive when the wet swab samples were negative, but no culture-positive wet swab samples were ever reported when the corresponding HEPA vacuum sock samples were negative. Results of HEPA vacuum sock and wipe samples were concordant 84% of the time; when they were discordant, the corresponding HEPA vacuum sock and wipe samples did not detect *B. anthracis* spores about the same number of times (five negative for HEPA vacuum sock and four negative wipe samples). Only the comparison of HEPA vacuum sock versus wipe sample had a Cohen's Kappa score >0.4, indicating fair to good agreement (Table 3).

The HEPA vacuum sock samples typically collected higher concentrations of *B. anthracis* spores than both the wet swab and wipe samples, and the wipe samples collected higher concentrations of spores than the wet swab samples (Table 4). These comparisons indicate good agreement between the HEPA vacuum sock samples and the wipe samples (Kendall's tau-b 0.66; Spearman's rank correlation coefficient 0.81).

Table 1. Sample summary statistics for *Bacillus anthracis* culture analysis, Brentwood Mail Processing and Distribution Center, December 17–19, 2001

Method	No. samples tested	*B. anthracis* detected (%)	Range[a] (CFU/cm^2)	Median[a] (CFU/cm^2)	Level[b]			
					Negative	Low	Medium	High
Dry swab	28	4 (14)	0.45–232.5	60.9	24	1	1	2
Wet swab	67	36 (54)	0.78–232.5	15.5	31	4	14	18
HEPA vacuum	64	51 (80)	0.3–81,000	23.1	13	9	14	28
Wipe	67	58 (87)	0.02–232.5[c]	5.4	9	9	36	13

[a]Positive samples only.
[b]Level of *B. anthracis* (CFU/cm^2): negative = 0, low = 0.1–1.6, medium=1.7–15.5, and high=>15.5.
[c]232.5 CFU/cm^2 is the maximum value considered too numerous to count for a concentration; 300 CFU is the maximum value considered too numerous to count for a culture.

Table 2. Dry swab versus other sampling methods for 28 locations, Brentwood postal facility, December 17–19, 2001

	Dry swab					
	No. concordant samples[a]		No. discordant samples[b]		Correlation	
Method	Positive (%)	Negative (%)	Dry positive	Dry negative	r_s[c]	p value[d]
Wet swab	4 (14)	15 (54)	0	9	0.43	0.024
HEPA vacuum	4 (14)	5 (18)	0	19	0.21	0.282
Wipe	4 (14)	5 (18)	0	19	0.07	0.719

[a]Two samples from the same location are concordant if both positive or both negative for *Bacillus anthracis* spores.
[b]Two samples from the same location are discordant if one is positive and the other negative for *B. anthracis* spores.
[c]r_s denotes Spearman's correlation coefficient between level of *B. anthracis* (CFU/cm^2) obtained by using the dry swab method and the level of *B. anthracis* obtained by using the comparison sampling method.
[d]p value for null hypothesis of zero correlation.

Although wet swabs were correlated with both the HEPA vacuum samples and the wipe samples, the agreement was not as strong.

The randomly selected surface areas where 13 HEPA vacuum sock samples had been collected were immediately sampled again with wipe samples. All the HEPA samples were positive for *B. anthracis* spores ranging in concentrations from 0.5 to 310 CFU/cm^2. The spore concentrations collected by the subsequent wipe samples (0 to 16 CFU/cm^2) were usually lower than the original vacuum samples; only two of the subsequent wipe samples were negative for *B. anthracis* spores.

The surface areas where 12 wipe samples were collected, corresponding to 12 of 13 HEPA vacuum sock samples, were immediately sampled again with HEPA vacuum sock samples. All the wipe samples were positive for *B. anthracis* spores, ranging in concentrations from 1.4 to 233 CFU/cm^2. Only one of the subsequent HEPA vacuum samples was negative for spores and the concentrations in nine of the HEPA vacuum sock samples were virtually the same as on the original wipe samples.

Discussion

The results of the side-by-side comparison of swab, wipe, and HEPA vacuum sock samples on nonporous surfaces indicated good agreement between the HEPA vacuum sock and wipe samples. However, the HEPA vacuum sock and wipe samples agreed poorly with the swab samples. The wet swabs did not detect spores >33% of the time when spores were detected by the wipe and vacuum sock samples. The dry swabs performed especially poorly, failing to detect spores >66% of the time when spores were detected by wipe and vacuum sock samples. Based on these results, dry swabs should not be used to sample for *B. anthracis* environmental contamination. Applying wet swabs in certain circumstances may be useful, for example, to sample crevices, inside machinery, and places difficult to reach by wipe and HEPA vacuum samples; however, dry swabs should not be used to sample surfaces where wipe and HEPA vacuum samples are likely to yield superior results. Sampling with wipes and HEPA vacuum socks is likely to yield very similar results on nonporous surfaces; wipes are preferable for sampling surfaces with relatively light dust, while HEPA vacuum socks should be selected to sample surfaces with heavy dust. Wipes may become quickly overloaded on dusty surfaces and thus unable to cover a large surface area. The sampling sensitivity of HEPA vacuum socks may be greater because they can collect large dust loads over much larger surface areas than wipes.

The relative difference between the wipe samples and the subsequent HEPA vacuum sock samples was not influenced by the initial concentration of spores collected by the wipe samples. After especially dirty areas were sampled with both wipes and HEPA vacuum sock samples, residual dirt was often still visible.

The samples were collected side by side so that the exact same surface area was not sampled by all methods. Because of nonuniform distribution, spore concentrations may have varied across the surfaces sampled by each method. However, we also set the order of sampling as random, making it unlikely that any particular method consistently encountered fewer spores than the other methods. Strong differences in these particular results more likely resulted from the sampling technique and not to nonuniform distribution of spores on these highly contaminated surfaces, where the different types of samples were collected very close to each other.

Table 3. Comparison of wet swab, wipe, and HEPA vacuum sock sampling methods, Brentwood postal facility, December 17–19, 2001

		No. concordant samples[a]		No. discordant samples[b]		
Methods compared	No. samples	Positive (%)	Negative (%)	Positive method	Positive method	Cohen's Kappa
Wet swab vs. wipe	67	35 (52)	8 (12)	Wet swab = 1	Wipe = 23	0.24
Wet swab vs. HEPA vacuum	58	27 (47)	10 (17)	Wet swab = 0	HEPA vacuum = 21	0.31
Wipe vs. HEPA vacuum	58	44 (76)	5 (9)	Wipe = 5	HEPA vacuum = 4	0.43

[a]Two samples from the same location are concordant if both positive or both negative for presence of *Bacillus anthracis* spores.
[b]Two samples from the same location are discordant if one is positive and the other negative for presence of *B. anthracis* spores.

Table 4. Comparison of *Bacillus anthracis* spore concentration levels in wet swab, wipe, and HEPA vacuum sock samples, Brentwood Mail Processing and Distribution Center, December 17–19, 2001

Comparison of concentration levels	HEPA vacuum vs. wet swab (n=58)		HEPA vacuum vs. wipe (n=58)		Wet swab vs. wipe (n=67)	
Levels[a] agree[b]	22	38%	26	45%	24	36%
Negative	10		5		8	
Low	1		2		0	
Medium	0		8		10	
High	11		11		6	
Levels disagree	36	62%	32	55%	43	64%
Higher levels	34	HEPA vacuum	23	HEPA vacuum	13	Wet swab
Higher levels	2	Wet swab	9	Wipe	30	Wipe
Kendall's tau-b	0.58		0.66		0.47	
Spearman's correlation r_s (p value)[c]	0.73 (<0.0001)		0.81 (<0.0001)		0.52 (<0.0001)	

[a]Level of *B. anthracis* (CFU/cm^2): negative = 0, low = 0.1–1.6, medium=1.7–15.5, and high=>15.5.
[b]Two samples from the same location agree if they are concordant and are both in the same grouping.
[c]p value for null hypothesis of zero correlation.

In areas likely to have been contaminated over a broad surface at high concentrations (such as DBCS machine no. 17), an adequate number of spores for detection was likely available for all three sampling techniques, but in other, less-contaminated areas, fewer spores were available for detection. Surface sampling clearly has inherent limitations. If investigators are careful to avoid contamination of the samples, the number of false-positive samples is reduced. However, sampling all surfaces within a building is not practical, and some surfaces containing *B. anthracis* spores might be missed.

The measurements collected in this study were not adequate to evaluate the sampling efficiencies of wipe and HEPA vacuum sock samples, particularly since the initial concentrations of spores on the sampled surfaces were unknown. However, sequential HEPA vacuum sock samples indicated better collection efficiency on nonporous surfaces than wipe samples. This efficiency is evident because wipe samples collected following vacuum samples were much lower than the initial vacuum samples, while the vacuum samples collected after wipe samples often collected a similar concentration of spores as the initial wipe samples. Care was taken after sampling to stay within the previously sampled area, but spores from outside the previously sampled area may have been inadvertently collected by the HEPA vacuum samples (e.g., spores from surrounding unsampled areas may have been drawn into the HEPA vacuum sock).

To avoid contamination of the vacuum when collecting samples, using disposable inserts may be more appropriate, such as cardboard sleeves, which can be placed inside the vacuum nozzle; the sampling sock can then be inserted into the sleeve and discarded after sampling. These sleeves should be discarded after sampling. Disposable inserts may prevent cross-contamination of the vacuum nozzle or subsequent sock samples. Care must be taken to prevent contamination of the inserts before they are used for sampling. While vacuum nozzles may not always be completely cleaned after sampling, our investigation indicated that cross-contamination could not be the reason for the high concentrations of spores detected on the numerous HEPA vacuum sock samples.

The results of this investigation may be used to guide future sampling efforts and serve as a baseline for follow-up measurements after the building has been cleaned further. The sampling and analytical techniques used in our study may provide useful reference for evaluations of other situations in the future. This study provides additional evidence for the need to quantify sampling efficiency to develop the type of limit-of-detection data normally created for other types of sampling and analytical methods. The collection efficiency (removing spores from the surface) and recovery efficiency (removing spores from the sampling media) need to be further evaluated for these methods. Our study focused on sampling nonporous surfaces; under these circumstances, HEPA vacuum sock samples and wipe samples performed similarly. However, this level of agreement may be difficult to achieve in sampling porous materials such as carpet and furniture, and the collection efficiency of sampling methods on other surfaces needs to be evaluated. Understanding the sampling efficiency of these methods on various types of surfaces is a critical requirement for future efforts to develop numerical criteria for surface contamination and potential exposures to humans. Lack of understanding about the efficiency of various sampling methods limits our ability to determine whether an environment has been adequately cleaned.

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References

1. Centers for Disease Control and Prevention. Evaluation of *Bacillus anthracis* contamination inside the Brentwood Mail Processing and Distribution Center B District of Columbia, October 2001. MMWR Morb Mortal Wkly Rep 2001:50:1129–33.
2. Angelotti R, Foter M, Busch K, Lewis K. A comparative evaluation of methods for determining the bacterial contamination of surfaces. Food Research 1958:23:175–85.
3. Angelotti R, Wilson J, Litsky W, Walter W. Comparative evaluation of the cotton swab and Rodac methods for the recovery of *Bacillus subtilis* spore contamination from stainless steel surfaces. Health Lab Sci 1964;1:289–96.
4. Favero M, McDade J, Robertson J, Hoffman R, Edwards R. Microbiological sampling of surfaces. J Appl Bacteriol 1968;31:336–43.
5. Whitfield W, Beakley J, Dugan V, Hughes L, Morris M, McDade J. Vacuum probe: new approach to the microbial sampling of surfaces. Appl Microbiol 1969;17:164–8.
6. Kirschner L, Puleo J. Wipe-rinse technique for quantitating microbial contamination on large surfaces. Appl Environ Microbiol 1979;38:466–70.
7. Buttner M, Cruz-Perez P, Stetzenbach L. Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR. Appl Environ Microbiol 2001;67:2564–70.
8. Dull PM, Wilson KE, Kournikakis B, Whitney EAS, Boulet CA, Ho JYW, et al. Bacillus anthracis aerosolization associated with a contaminated mail sorting machine. Emerg Infect Dis 2002;8;1044-7.
9. Centers for Disease Control and Prevention. Comprehensive procedures for collecting environmental samples for culturing *Bacillus anthracis*. Available from: URL: http://www.bt.cdc.gov/Agent/environmental-sampling-apr2002.asp . Accessed May 10, 2002.
10. Alcamo I. Fundamentals of microbiology. Sudbury, Massachusetts: Jones and Bartlett Publishers; 2000. p. 710–1.
11. Centers for Disease Control and Prevention. American Society for Microbiology, Association of Public Health Laboratories. Basic diagnostic testing protocols for Level A laboratories for the presumptive identification of *Bacillus anthracis*. Available from: URL: http://www.asmusa.org/pcsrc/ban.asm.la.cp.102401f.pdf . Accessed October 2001.
12. Logan N, Turnbull P. Bacillus and recently derived genera. In: Murray P, Baron E, Pfaller M, Tenover F, Yolken R, editors. Manual of clinical microbiology. 7th edition. Washington, D.C.: American Society for Microbiology; 1999. p. 357–69.
13. Khan A, Morse S, Lillibridge S. Public health preparedness for biological terrorism in the USA. Lancet 2000:356;1179–82.
14. SAS Institute, Inc. SAS/STAT user's guide, version 8. Cary, NC: SAS Institute, Inc.; 1999. p. 3884.
15. Fleiss JL. Statistical methods for rates and proportions. 2nd edition, New York: John Wiley & Sons, Inc.; 1981. p. 218.
16. Hollander M, Wolfe DA. Nonparametric statistical methods. 2nd edition. New York: John Wiley & Sons, Inc.; 1999. p. 394–408.
17. Blalock H. Social statistics. New York: McGraw-Hill Co.; 1979. p. 436–9.

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Collaboration between Public Health and Law Enforcement: New Paradigms and Partnerships for Bioterrorism Planning and Response

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The biological attacks with powders containing *Bacillus anthracis* sent through the mail during September and October 2001 led to unprecedented public health and law enforcement investigations, which involved thousands of investigators from federal, state, and local agencies. Following recognition of the first cases of anthrax in Florida in early October 2001, investigators from the Centers for Disease Control and Prevention (CDC) and the Federal Bureau of Investigation (FBI) were mobilized to assist investigators from state and local public health and law enforcement agencies. Although public health and criminal investigations have been conducted in concert in the past, the response to the anthrax attacks required close collaboration because of the immediate and ongoing threat to public safety. We describe the collaborations between CDC and FBI during the investigation of the 2001 anthrax attacks and highlight the challenges and successes of public health and law enforcement collaborations in general.

Public health and law enforcement agencies become involved in the investigation of a possible bioterrorism event under different circumstances. Such events fall into one of two categories: overt and covert. In the overt event, the perpetrator announces responsibility for something (for example, release of an agent) or the nature of the event reveals itself (i.e., the 1995 sarin attack by the Aum Shinrikyo in the Tokyo subway). In the overt attack, usually law enforcement first detects the event, leads the initial response, and notifies public health officials (Figure 1). If persons are ill or preventive health services are indicated, public health will also become involved in the emergency response.

In contrast, the covert event is characterized by an unannounced or unrecognized release in which the presence of ill persons may be the first sign of an attack. In the covert attack, criminal intent may not be apparent until some time after illnesses are recognized. This distinction is important for establishing and understanding the partnership between public health and law enforcement. The overt event is clearly a crime, and the site of the incident is a crime scene. As a result, access to the area may be restricted so that evidence can be collected pursuant to the criminal investigation. Under federal statute (Title 18, U.S.C. Section 2332[a]), any threatened use of a disease-causing organism directed at humans, animals, or plants is a crime, regardless of whether the perpetrator actually possesses a disease-causing agent. In addition, as a result of a change in the Bioterrorism Weapons Anti-Terrorism Act contained in the USA PATRIOT Act of 2001 and codified in Title 18 USC Section 175(b), knowingly possessing a biological agent, toxin, or delivery system which cannot be "justified by a prophylactic, protective, bona fide research, or other peaceful purpose" can result in arrest, prosecution, and fines and/or imprisonment for up to 10 years. This new provision shifts the burden of proof onto the person or persons who are in possession of dangerous biological agents to prove they have the material for legitimate purposes.

The covert event may not be initially recognized as an attack, and public health generally first recognizes the problem and leads the initial inquiry (Figure 2). The early response will focus on diagnosis, medical care, and epidemiologic investigation. The intentional and criminal nature of the event may not be immediately evident, and notification of law enforcement may be delayed as a result. A 1985 outbreak of gastroenteritis in Oregon that was caused by a religious cult contaminating multiple salad bars with salmonella was initially thought to be a natural event (1). The crime was only recognized after the cult's leader accused other cult members of the attack and publicly called for an investigation. The subsequent criminal investigation confirmed the role of cult members in the outbreak.

Microbiologic factors may also provide the first clue of the criminal intent of a disease outbreak. In 1996, an outbreak of gastroenteritis among staff in the laboratory of a large medical center was caused by *Shigella dysenteriae* type 2, a pathogen that is unusual in the United States (2). An epidemiologic investigation linked infection with eating pastries that had been placed in the laboratory break room. *S. dysenteriae* type 2 matching the laboratory's stock strain by pulsed-field gel elec-

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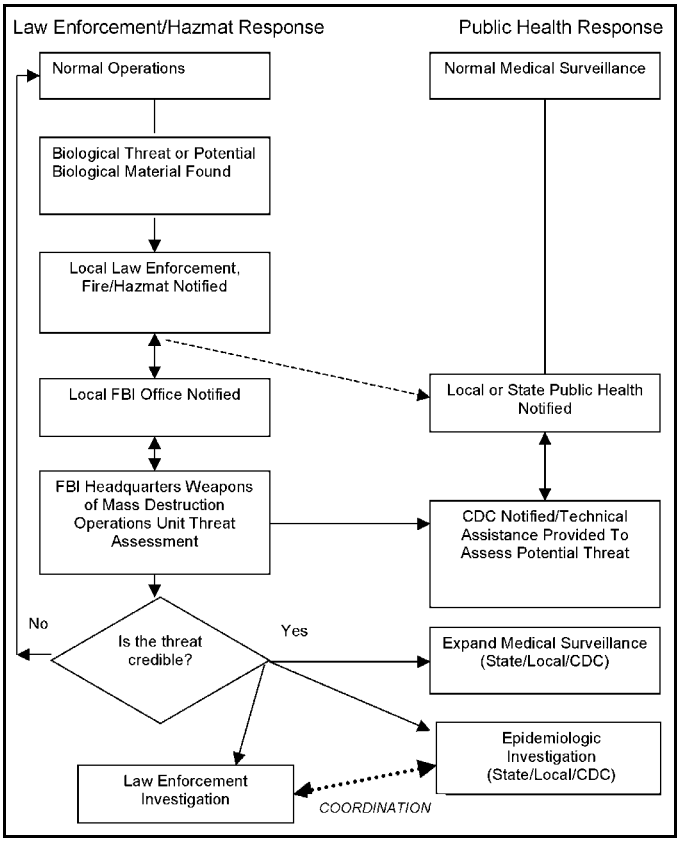

Figure 1. Likely flow of communication during overt bioterrorism in most (solid line) and some (dashed line) jurisdictions. HAZMAT, hazardous materials management personnel.

trophoresis was recovered from ill laboratory workers and from an uneaten pastry. A portion of the laboratory's stock strains was missing, and subsequent criminal investigation identified a disgruntled former laboratory employee as the perpetrator.

The anthrax attacks in September and October 2001 provide examples of both overt and covert events and highlight the different ways that public health and law enforcement agencies become involved in investigating bioterrorist attacks. The first case that was recognized in Florida in early October could have represented a natural event and was initially investigated as a public health issue (3,4). However, law enforcement officials were notified and involved in the initial investigation because of the rarity of inhalational anthrax in the United States (5,6), because *B. anthracis* has known potential as a biological weapon (7,8), and because of increased vigilance for a possible bioterrorist attack after the events of September 11. Once the intentional nature of the event was made evident by the second suspected case of inhalational anthrax in Florida, law enforcement involvement increased dramatically. The receipt of an envelope containing a threatening letter and *B. anthracis* at the Hart Senate Office Building on October 15, 2001, required that the site be handled as a crime scene, and the intial role of public health was primarily consequence management and technical assistance to the Federal Bureau of Investigation (FBI) and other law enforcement officials.

Similarities and Differences

Although both public health and law enforcement protect the public, the approach and nature of the work performed in the two disciplines are quite different. The similarities and differences in public health and law enforcement investigations have to be understood and coordinated so that both can be most effective (Table). Public health investigations generally take an inductive approach. Persons are interviewed, data are collected, hypotheses are developed to explain transmission, and epidemiologic and laboratory studies are conducted to test these hypotheses. If the studies confirm the hypothesis, prevention and control strategies are developed, implemented, and evaluated. All this work is held to the standard of scientific peer review, generally through presentation of data at scientific meetings and publication in a scientific journal.

On the other hand, the law enforcement investigation takes a deductive approach and is held to a very different standard. Witnesses and potential suspects are interviewed, leads are developed and pursued, and all available evidence is collected, identified, and tracked. If evidence is adequate, the suspected perpetrator is identified, arrested, and prosecuted. The work of law enforcement is held to legal standards. Thus, while the public health investigator's aim is to collect data that will withstand the scrutiny of subject matter experts and the global scientific community, with the ultimate goal of developing effective control measures, the law enforcement investigator's

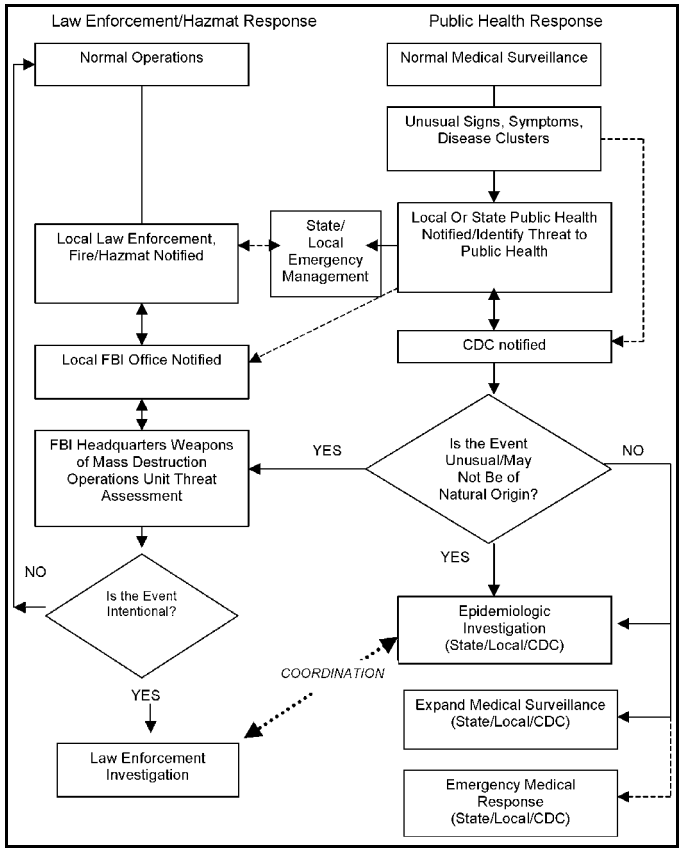

Figure 2. Likely flow of communication during covert bioterrorism in most (solid line) and some (dashed line) jurisdictions. HAZMAT, hazardous materials management personnel.

Table. Differences in public health and law enforcement investigations

Characteristics	Public health	Law enforcement
Method of event recognition	Event detected through public health surveillance or calls from clinicians	Event announced by attacker or is evident
Challenges to event recognition	Few clinical syndromes that are clearly the result of bioterrorist attack; difficulty distinguishing between disease of natural origin and bioterrorism attack	Large number of hoaxes and noncredible threats not associated with an actual bioterrorist attack; delay in notification of possible event by public health; "copycat" threats or attacks (9)
Initial data collection	Hypothesis generation, "shoe-leather epidemiology"	Questioning of witnesses and suspects, follow-up of tips and intelligence information
Confirmatory data collection and analysis	Controlled epidemiologic studies	Collection and organization of evidence
Data validation	Presentation for scientific peer review	Indictment, arrest, and conviction
Goal of investigation	Effective disease prevention and control measures	Prevention and deterrence of future attacks

goal is gathering evidence that will meet constitutional standards and withstand legal challenges to obtain a conviction.

The differing nature of the work and standards to which the work is held can pose difficulties on occasion when public health and law enforcement officials conduct joint investigations. In high-profile investigations, such as the anthrax attacks in 2001, these differences can be exaggerated by public perceptions and media portrayals of public health and law-enforcement investigative methods. The issues become even more complex when events involve multiple geographic areas or organizations that have overlapping responsibilities. These difficulties can be addressed within the public health and law enforcement communities by understanding each other's approaches, by communicating effectively, and by making thoughtful preparations, including testing the system through exercises (10–12). These measures will improve collaboration during crises. The adage that "an emergency is a bad time to begin exchanging business cards" applies. During the investigations of the anthrax attacks in 2001, preexisting relationships between FBI field offices and state and local public health officials improved communications for field investigations and facilitated the public health response (M. Layton, New York City Health Department, pers. comm.).

Preexisting relationships were particularly important for coordinating microbiologic testing of environmental and clinical samples, which were critical to both investigations. Before the 2001 anthrax incidents, the Centers for Disease Control and Prevention (CDC) and FBI began working together to develop notification procedures for possible bioterrorism events and to establish the Laboratory Response Network (LRN) for Bioterrorism, a multilevel network connecting local and state public health laboratories with advanced capacity public health and military laboratories (13). The federal, state, and local collaborative effort of law enforcement and public health that developed the LRN is the result of predicting the need for validated tests that would be consistent with evidentiary requirements. A uniform set of laboratory protocols, based on established procedures and reagents, facilitates the introduction of test results into a court of law, thereby limiting evidentiary challenges that may result from the use of different testing methods or analyses. Because clinical specimens are referred to LRN laboratories for analysis, the LRN also serves as a front-line resource and detection mechanism for identifying a potential covert attack. The 2001 anthrax incidents demonstrated the importance of the LRN in responding to a biological attack and revealed the need to expand its laboratory capacities.

New Partnerships, New Paradigms

Although federal, state, and local public health plans for responding to bioterrorism contributed to a state of readiness that would not have been possible only a few years earlier, the response to the 2001 anthrax attacks required venturing into unfamiliar territory for many public health and law enforcement officials. Historically, most terrorist attacks on Americans have involved use of explosives (14), and investigations have been conducted by FBI and other law enforcement agencies, while public health involvement has generally been limited to ensuring safe working conditions for investigators and aid workers and assessment of the acute and long-term physical and mental health effects (15–19).

For many public health officials, responding to the rising threat of bioterrorism and recent attacks has necessitated a steep learning curve. Public health investigators usually approach infectious disease outbreaks as naturally occurring events, rather than the result of criminal acts, and they are unaccustomed to working closely with law enforcement personnel (11,12). Additionally, national security clearance has not been a requirement for most public health professionals, for whom the clearance process is unfamiliar. During 2001, few public health investigators had equipment such as secure telephone and fax lines necessary for sharing sensitive information with law enforcement officials. Confidentiality is maintained in public health investigations for the purpose of protecting sensitive patient medical information rather than national security. In law enforcement, confidentiality is also maintained to protect informants and witnesses and to preserve the integrity of the case for prosecution. Before 2001, most public health officials were not familiar with the principles of maintaining the chain of custody of specimens submitted for microbiologic testing so that laboratory results could be used for criminal prosecution.

Collaboration with law enforcement officials generally has not been recognized as beneficial or desirable in public health. The presence of law enforcement officers has been thought to compromise the collection of sensitive medical information (e.g., illegal drug use). Indeed, some degree of separation from law enforcement may be advantageous for obtaining complete and accurate data during public health investigations. Public health services are vitally needed by medically underserved communities, where suspicion of law enforcement agencies is intense, and collaboration with law enforcement agencies has even been described as "destructive to public health efforts" (20). However, the role of law enforcement in investigating potential bioterrorism incidents requires interviewing all potential witnesses and victims. Separate questioning by law enforcement and public health investigators may lead to conflicting statements by the interviewee, jeopardizing the admissibility of those statements in subsequent judicial proceedings. A process should be established whereby joint interviews by public health and law enforcement officials are conducted, with opportunity for confidential communications with public health officials regarding specific health-related issues that the interviewee may be unwilling to share with law enforcement personnel present. Both law enforcement and public health must recognize that the sharing of information can be crucial for identifying persons who have been exposed to dangerous agents and may be in need of prevention services such as chemoprophylaxis or vaccination.

Law enforcement is now increasingly focused on prevention of terrorist acts, requiring a new partnership with the public health and medical community. The steps necessary to identify a potential covert bioterrorism attack include a close coordination between those who collect and analyze medical and syndromic surveillance information with the law-enforcement community's intelligence and case-related information. The best method for timely detection of a covert bioterrorist attack is early communication between the two communities and recognition of the extent and origin of the threat. For the FBI, this recognition requires conducting a threat/credibility assessment, a process coordinated by the Weapons of Mass Destruction Operations Unit, FBI Headquarters, in conjunction with CDC and other federal agency experts. The FBI threat assessment is necessary to determine whether the circumstances may be the result of an intentional or criminal act, warranting law enforcement involvement. In some cases, a joint FBI–public health investigation is necessary to gather facts to determine whether a criminal act has actually occurred.

The work of CDC and FBI during the ongoing anthrax investigation highlights the opportunity for collaboration between public health and law enforcement. During several of the anthrax field investigations in 2001, investigators from FBI or local law enforcement were paired with an epidemiologist during interviews of possible case-patients and exposed persons, which allowed a multidisciplinary approach to collecting, processing, and sharing pertinent information. Because of different training backgrounds and professional experiences, law enforcement and public health interviewers may recognize and note different information or clues that could aid in identifying the source of the infection and its perpetrator(s). Additionally, the concurrent interviews reduced the number of times persons had to be questioned. Since October 12, 2001, a senior medical epidemiologist from the National Center for Infectious Diseases, CDC, has been assigned to FBI headquarters or to the Washington field office to help facilitate communication of information between the agencies and to provide on-site medical and public health consultation as threats of new possible biological attacks are assessed.

Conclusion

Partnership between public health and law enforcement is prerequisite to sound bioterrorism planning and response. Each group can add value to the work of the other. At the federal level, both CDC and FBI have unique perspectives and expertise that can benefit the other. For the FBI, CDC offers medical and laboratory consultation and collaboration combined with national and international public health connections. For CDC, the FBI offers criminology expertise, forensic laboratory collaboration, and access to intelligence information, along with national and international law enforcement connections. Each agency offers a unique perspective and opportunities to share information. Similar partnerships exist or should exist at the state and local level. Public health and law enforcement must understand each other's work, standards, and culture. The heat of an investigation can strain even the best relationships. Thus, public health and law enforcement need to increase mutual collaboration and understanding before they are thrown together in the response to a biological attack. To this end, liaison personnel are needed who have some degree of cross-training in the public health aspects of communicable diseases and in law enforcement and criminal investigations.

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References

1. Török TJ, Tauxe RV, Wise RP, Livengood JR, Sokolow R, Mauvais S, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. JAMA 1997;278:389–95.
2. Kolavic SA, Kimura A, Simons SL, Slutsker L, Barth S, Haley CE. An outbreak of *Shigella dysenteria* type 2 among laboratory workers due to intentional food contamination. JAMA 1997;278:396–8.
3. Centers for Disease Control and Prevention. Notice to readers: ongoing investigation of anthrax–Florida, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:877.

4. Bush LM, Abrams BH, Beall A, Johnson CC. Index case of fatal inhalational anthrax due to bioterrorism in the United States. N Engl J Med 2001;345:1607–10.
5. Brachman PS. Bioterrorism: an update with a focus on anthrax. Am J Epidemiol 2002;11:981–7.
6. Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Anthrax as a biological weapon, 2002. Updated recommendations for management. JAMA 2002;287:2236–52.
7. Henderson DA. Bioterrorism as a public health threat. Emerg Infect Dis 1998;4:488–92.
8. Kortepeter MG, Parker GW. Potential biological weapons threats. Emerg Infect Dis 1999;5:523–7.
9. Stern J. The prospect of domestic bioterrorism. Emerg Infect Dis 1999;5:517–22.
10. Inglesby TV, Grossman R, O'Toole T. A plague on your city: observations from TOPOFF. Clin Infect Dis 2001;32:436–45.
11. Hoffman RE, Norton JE. Lessons learned from a full-scale bioterrorism exercise. Emerg Infect Dis 2000;6:652–3.
12. O'Toole T, Mair M, Inglesby TV. Shining light on A "Dark Winter." Clin Infect Dis 2002;34:972–83.
13. Khan AS, Morse S, Lillibridge S. Public-health preparedness for biological terrorism in the USA. Lancet 2000;356:1179–82.
14. Department of Justice. Terrorism in the United States, 1999. Washington, DC: Counterterrorism Threat Assessment and Warning Unit, Counterterrorism Division; 1999. Federal Bureau of Investigation pub. No. 0308.
15. Quenemoen LE, Davis YM, Malilay J, Sinks T, Noji EK, Klitzman S. The World Trade Center bombing: injury prevention strategies for high-rise building fires. Disasters 1996;20:125–32.
16. Mallonee S, Shariat S, Stennies G, Waxweiller R, Hogan D, Jordan F. Physical injuries and fatalities resulting from the Oklahoma City bombing. JAMA 1996;276:382–7.
17. Dellinger AM, Waxweiller RJ, Mallonee S. Injuries to rescue workers following the Oklahoma City bombing. Am J Indust Med 1997;31:727–32.
18. North CS, Nixon SJ, Shariat S, McMillen JC, Spitznagel EL, Smith EM. Psychiatric disorders among survivors of the Oklahoma City bombing. JAMA 1999;282:755–62.
19. Hogan DE, Waeckerle JF, Dire DJ, Lillibridge SR. Emergency department impact of the Oklahoma City bombing. Ann Emerg Med 1999;34: 160–7.
20. Sidel VW, Cohen HW, Gould RM. Good intentions and the road to bioterrorism preparedness. Am J Public Health 2001;91:716–8.

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Collaboration between Public Health and Law Enforcement: The Constitutional Challenge

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In their article "Collaboration between Public Health and Law Enforcement: New Paradigms and Partnerships for Bioterrorism Planning and Response," Butler et al. present a valuable introduction to the practical problems of coordinating public health and criminal law investigations (1). While this problem is not new in public health, the events of September 11, 2001, have given it a special urgency. This commentary outlines the constitutional constraints on such collaborations, with the objective of helping public health and law-enforcement personnel resolve issues that are not addressed by the article.

Constitutional Limitations

The Constitution is the source of all legal authority in the United States. Written in 1789, the Constitution was shaped by the events of the time. The weak union of the Articles of Confederation made it difficult to wage the Revolutionary War, so the Constitution provided a strong central government with the power to wage war and raise revenue directly, without depending on state legislatures. The abuses of power by English colonial governors led to the Constitution's Bill of Rights, which strictly limits the state's powers to prosecute and punish individuals for violating the laws—the criminal law power—and to seize personal property for governmental use—the takings power.

At the same time, the terrible toll exacted on the colonies by epidemic disease (2) led the drafters to allow the states very broad powers to abate nuisances and regulate other threats to the public health. The public health authority is known as the police powers (3), as in "to police," meaning to clean up. Formal law-enforcement departments were formed several years after the ratification of the Constitution. Originally, these departments had broader responsibilities than do modern law-enforcement agencies, including some public health functions, so calling them police forces was more consistent with their original function than their current one.

Under the criminal law power, persons who are accused of crimes 1) may not be subjected to search and seizures without probable cause; 2) may not be forced to incriminate themselves; 3) are entitled to a jury trial; 4) are entitled to legal counsel if they are indigent; 5) are entitled to have the case against them proved beyond a reasonable doubt; 6) must be prosecuted under a law that clearly identifies the forbidden behavior; and 7) generally have extensive due process rights to assure that they are not improperly imprisoned. Under the takings power, persons whose property is being seized for the public good have the right to a court hearing and to fair market compensation for the property. In contrast, under the police power, public health officials 1) may search and seize without probable-cause warrants; 2) may take enforcement actions without prior court hearings; 3) are entitled to have courts defer to their discretion; 4) have great flexibility in crafting enforcement strategies; and 5) must only prove their cases by a "more probable than not" standard if the actions are challenged in court (4).

From the ratification of the Constitution to the present day, tension has existed between the Bill of Rights and the police powers. In a key precedent case, a health department seized and destroyed 47 barrels of contaminated poultry from a cold storage plant. The owners claimed that they had been denied due process and just compensation for the value of the property. The court ruled that the destruction or regulation of threats to the public health entitled the owner only to minimal due process and no compensation (5). Other cases established that persons who threaten the public health could be quarantined or subjected to other limitations on their liberty without triggering criminal law due process requirements. Recent cases have concerned whether land use regulations that prevent construction are an improper taking (6) and whether closing bathhouses violates the right of free association (7).

From the earliest cases, the courts have recognized that the public health powers, defined too broadly, would undermine the Bill of Rights. The courts demand that the state demonstrate that the action ordered is intended to prevent harm in the future, not to punish for past actions, and that the action is reasonably related to the public health objective. A gonorrhea control program that involved the temporary detention of prostitutes until they could be examined or treated for gonorrhea (8) was found constitutional (9) because the detention was not a punishment and prostitutes were shown to be an important factor in the spread of gonorrhea in the community (10). A fire ordinance that applied only to Chinese-owned laundries was found unconstitutional because it was not rationally related to preventing fires and was thus an impermissible race law (11).

The courts recognize that there is a continuum between pure public health laws and criminal laws. The more closely the action approaches a criminal punishment, the more protection the individual is entitled to. Thus, mental health commitments,

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which have a public health component but also resemble imprisonment, require more due process protections than does a quarantine order, but fewer protections than a criminal prosecution. Preventive detention of accused criminals, such as mobsters who might kill witnesses, most closely resembles imprisonment for punishment and must be done with almost the same level of due process as a prosecution (12).

The United States Supreme Court and other courts that decide cases based on U.S. Constitutional law give great deference to state laws dealing with communicable disease control and sanitation, the key issues in bioterrorism. Historically, most state courts, construing their own state constitutions, have allowed public health officials the same latitude and discretion for disease control and sanitation as the U.S. Constitution. However, in the 1980s and 1990s, several states revised their communicable disease control laws or passed special AIDS laws that greatly restricted the authority of public health officials. Some of these laws were subsequently revised because they made it impossible to carry out tuberculosis control programs, but many might still interfere with a bioterrorism investigation. While most states allow food inspectors and other sanitary inspectors broad powers in theory, many state laws divide these powers among several agencies, making prompt and effective investigations almost impossible.

Bioterrorism Investigations

Certain public health functions, such as sexually transmitted diseases (STD) control, have always involved cooperation with the police. In such situations, public health officials usually do not pass on information to the police that would result in the arrest of infected persons for related crimes such as prostitution. Yet even STD clinics report potential child abuse and provide information to law enforcement to assist their investigations. Bioterrorism investigations require close cooperation between public health and law enforcement, which entails some blurring of their usual roles. Public health investigators will function as forensic experts if there is a prosecution, and law enforcement will try to prevent the further spread of disease by identifying and arresting the perpetrators.

Public health officials can respond quickly to an identified threat and can conduct investigations without the limitations of probable-cause warrants. Public health officials cannot use their powers to circumvent the criminal law protections provided by the Constitution (13). Information gained from public health investigations that do not meet criminal due process standards cannot be used in criminal prosecutions, and if such information is relied on by the police, it may contaminate their subsequent investigations and render all their evidence inadmissible.

Information from public health investigations may be used in criminal investigations if two criteria are met. First, the information must be collected and processed with a proper chain of custody so that it can be authenticated by an expert and admitted into evidence. Since careful handling is also critical to proper epidemiologic investigations, this standard of care should be maintained in all investigations. Second, the evidence must be obtained as part of a legitimate public health investigation. For example, food samples taken during an investigation of food poisoning at a picnic could be used in a subsequent criminal trial if the food was found to be intentionally contaminated. In contrast, food inspectors cannot use their authority to inspect a restaurant kitchen as a pretext for searching the lockers of restaurant employees. If evidence were found in an employee's locker, a judge would not be likely to allow it to be admitted in a criminal prosecution of the employee. To be admissible, a law-enforcement officer would need to obtain a search warrant from a judge before searching the lockers. This necessity could delay the search and might raise public health issues if it was feared that a toxic substance was leaking from the locker and endangering the public. Such conflicts between purposes would be much more severe for an agent such as smallpox, for which decontamination to protect the public might destroy all evidence at the site.

From the perspective of law enforcement, all investigations should be done under criminal law standards to ensure that the perpetrators will be punished at the conclusion of the investigation. To a great extent this coordination was possible in the recent anthrax investigations because the event was discovered after the initial exposure and there was no risk of person-to-person spread. In the case of an evolving epidemic of a more communicable agent, it may be necessary to choose between protecting the population and collecting evidence that will be admissible in criminal investigations. Public health and law-enforcement agencies can minimize this potential conflict by careful planning, as outlined in Butler et al. (1). In many states, the public health inspection laws should be harmonized to assure that a team can be quickly assembled with the authority to conduct all necessary inspections, whether they involve restaurants, workplaces, food processing plants, or agribusiness enterprises. By clarifying legal authority before an incident occurs and increasing communication between government agencies, especially between forensic laboratories and the public health laboratories, the necessity to choose between public health and law enforcement can be lessened.

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References

1. 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;1152-6.
2. Powell JH. Bring out your dead: the great plague of yellow fever in Philadelphia in 1793. Philadelphia: University of Pennsylvania Press; 1949.
3. Freund E. The police power § 3. Chicago: Callaghan and Company; 1904. p. 3.

4. Richards EP, Rathbun KC. Public health law. In: Wallace RB, editor. Appleton and Lange Public health and preventive medicine. New York: McGraw-Hill; 1998. p. 1147–54.
5. *North American Cold Storage Co. v City of Chicago*, 211 U.S. 306 (1908).
6. *Tahoe-Sierra Preservation Council, Inc. v Tahoe Regional Planning Agency*, 122 SCt 1465 (2002).
7. *City of New York v. New St. Mark's Baths*, 130 Misc. 2d 911, 497 N.Y.S.2d 979 (N.Y. Sup. 1986).
8. Potterat JJ, Rothenberg RB, Muth JB, Woodhouse DE, Muth SQ. Invoking, monitoring, and relinquishing a public health power: the health hold order. Sex Transm Dis 1999;26:345–9.
9. *Reynolds v McNichols,* 488 F.2d 1378 (10th Cir. 1973).
10. Richards EP, Rathbun KC. The role of the police power in 21st century public health. Sex Transm Dis 1999;26:350–7.
11. *Yick Wo v Hopkins*, 118 U.S. 356 (1886).
12. *United States v. Salerno*, 481 U.S. 739 (1987).
13. Richards EP. The jurisprudence of prevention: society's right of self-defense against dangerous individuals. Hastings Constitutional Law Quarterly 1989;16:329–92.

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Epidemic Anthrax in the Eighteenth Century, the Americas

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Anthrax has been described as a veterinary disease of minor importance to clinical medicine, causing occasional occupational infections in single cases or clusters. Its potential for rapid and widespread epidemic transmission under natural circumstances has not been widely appreciated. A little-known 1770 epidemic that killed 15,000 people in Saint-Domingue (modern Haiti) was probably intestinal anthrax. The epidemic spread rapidly throughout the colony in association with consumption of uncooked beef. Large-scale, highly fatal epidemics of anthrax may occur under unusual but natural circumstances. Historical information may not only provide important clues about epidemic development but may also raise awareness about bioterrorism potential.

In late 2001, anthrax bioterrorist attacks in the United States prompted considerable commentary on how little is generally appreciated about the transmissibility of *Bacillus anthracis*. Textbooks have long described anthrax as a veterinary disease of minor medical importance, attributing most human infections to occupational exposures, now less common in industrialized nations. Because anthrax is usually recognized in single cases or small clusters, its potential for rapid and broad dissemination to humans under natural circumstances has not been widely appreciated. Such a potential would have implications for both epidemics and bioterrorism.[1]

An obscure report claims that an explosive 1770 epidemic of what was called *charbon* killed 15,000 persons in Saint-Domingue (modern Haiti). The brief description of this epidemic, written by historian Michel-Placide Justin (1), is unknown to most physicians and historians. The epidemic began shortly after an earthquake near Port-au-Prince on June 3, 1770, devastating the city and much of the western end of the island. With bakeries, stores, storehouses, and many or most of the buildings and homes in major towns destroyed, and with the consequent escape of slaves who typically obtained, transported, and prepared food for themselves and the colonists, famine was a serious threat. Trade regulations in force at the time specifically restricted importation of meats or salted fish. Justin describes the situation as follows:

"…The unfortunate slaves in the north of Saint-Domingue therefore experienced the most frightful famine. The dependencies of Fort Dauphin, that of Gros-Morne, [and] of Jean Rabel, were devastated. Codfish being entirely unavailable, the Spaniards, whose *hattes* [presumably a form of the Spanish "hato", meaning "cattle ranch"] or pastures were being thinned out daily by a terrible epizootic ["épizootie"], sought to salt or smoke all their ill or dead animals; and they [then] brought them into French establishments. These meats, known as *tassau* in the colonies, which the Negroes avoided eating when they could get [uncontaminated] salted beef and codfish, spread to the slaves the communicable agent ["germe"] of the disease with which they [the meats] were infected ["infectées"]. A type of epidemic disease ["peste"], called anthrax ["charbon"], spread throughout all the neighboring dwellings of the Spaniards or the routes they frequently used, and in those where the Negroes had bought this *tassau*. Within six weeks, more than 15,000 white and black colonists perished of this terrible disease, and its ravages did not stop until the government, the magistrates, and the inhabitants themselves had joined all of their efforts to repel the scourge introduced into the colony by Spanish greed.

"But the numerous and rapid deaths caused by the disease were not all: at least 15,000 Negroes perished of hunger, and the escape of slaves increased in the northern dependency, causing serious fear for the security of the colony…" (1)

Although sketchy, this report of possible epidemic anthrax contains interesting details. It notes the precipitating circumstances of an ongoing epizootic and a sudden change in diet to uncooked—smoked or salted—beef. The report also discloses that outbreak investigation linked the distribution of contaminated beef to the geographic spread of human disease. These associations appear consistent with intestinal anthrax, a disease associated with high mortality. However, exact means and determinants of gastrointestinal transmission were not described. Salted or smoked meat likely would have been eaten without cooking, as was then the custom. Since anthrax spores are resistant to 140°F and to a wide range of chemical treatments, the failure of salting or smoking to destroy them would not be surprising.

Apparently the overall mortality in the epidemic was high, although the figure of 15,000 deaths may have been only an

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[1]The intentional release of *Bacillus anthracis* in October 2001 greatly challenged the U.S. public health system. Collaborating with partners in other federal, state, and local health agencies, the Centers for Disease Control and Prevention (CDC) responded to these bioterrorism events (1) by relying on experience investigating public health aspects of anthrax over the past 50 years.

estimate. Vital events data from the epidemic were probably obtained by French officials and sent to Paris, but I am not aware that such data, if they still exist, have been identified by historians. Neither attack rates nor case-fatality rates have been documented from this or similar anthrax epidemics in the same era, although eighteenth-century observers were nearly unanimous in indicating a high or universal death rate from intestinal anthrax.

Had the epidemic occurred several years later, it might have received more thorough official attention. In 1776, Félix Vicq-d'Azyr set up a system of epidemic surveillance and outbreak investigation that operated in France and her colonies until 1794 (2). Two of its "correspondent" proto-epidemiologists, Drs. Arthaud and Girard, were in place in Saint-Domingue to report on epidemics by November 1777 and February 1778, respectively. Six years later, Saint-Domingue's *Cercle des Philadelphes*, of which Benjamin Franklin became a member, had been established and had also begun to study medical and veterinary diseases. Publications of this and other societies describe Haitian epidemics in the early 1770s, but none mention the 1770 outbreak.

The identity of the disease described in Justin's report can be questioned. I have translated the report's designation of *charbon* as "anthrax," the term corresponding to modern anthrax. But in 1770, 10 years before epizootic anthrax had been reasonably well described by Chabert (3) and 95 years before its microbial cause was fully demonstrated by Davaine (4), the term *charbon* ("charcoal") was sometimes applied nonspecifically to other human diseases producing skin lesions, including not only dark or violaceous lesions of any sort but also plague and smallpox. Justin's sources for the 1770 epidemic report are unknown, but he did not begin to write it until 1822 or 1823, by which time human and animal anthrax had become better understood.

Other possibilities for the cause of the epidemic seem less likely. Smallpox epidemics periodically swept Caribbean islands (e.g., Barbados in 1751, causing a serious case of smallpox in future U.S. president George Washington [5]). However, it was well known that smallpox did not cause epizootics in cattle, and French officials would not likely have mistaken such a familiar disease. Aside from its clear clinical picture, any epidemic that spared past smallpox victims would have been immediately noted by Europeans, all of whom knew their own status with regard to smallpox susceptibility. Moreover, by 1770 many colonists and slaves had been variolated, making recognition of epidemic smallpox even more likely.

In 1801, American proto-epidemiologist (and future lexicographer) Noah Webster speculated that the 1770 epidemic "must have been the real plague" (6), what we now call "bubonic plague." This speculation seems to have been based on his discovery during 1799–1801 of 30-year-old gazette accounts, which he did not, unfortunately, cite. Webster might also have been influenced by description of the disease as a type of "peste," a word which, in the 1770s, could mean either an epidemic disease of any kind or the specific disease now known as plague, caused by *Yersinia pestis*. However, neither bubonic nor pneumonic plague is consistent with a cattle epizootic or an association with beef distribution. A "fatal angina" or "distemper" (also described as a "sore throat") appears to have been epidemic in the Caribbean in 1770 (7,8), but in that era such terms usually indicated either diphtheria or streptococcal pharyngitis (9), neither of which causes fatal epizootics. Rabies was introduced into Saint-Domingue about 1776 (10) but seems entirely inconsistent on clinical and epidemiologic grounds.

In rare post-Webster medical references to the 1770 epidemic, anthrax has not been questioned. For example, a passing reference in a medical text by anthrax authority Carl von Heusinger (11), published in 1850, agrees on anthrax, a diagnosis subsequently accepted without comment in George Fleming's 1871–1882 history of epizootics (12) and in James Law's 1885 review of "malignant pustule" (13).

Also notable with regard to the epidemic's identity are 1775 reports claiming that a less severe epizootic of the same disease recurred in Saint-Domingue in 1772, spread to Guadeloupe, then recurred again in Saint-Domingue in 1773, 1774, and 1775. These subsequent epidemics affected cattle and caused, in humans, both cutaneous lesions, associated with inoculation, and gastrointestinal diseases, associated with ingestion (10,14). These reports and others published by members of the *Cercle des Philadelphes* appear to be excellent early descriptions of anthrax. The author of one of them (14), proto-epidemiologist Charles Arthaud, sent information from the 1774–75 epizootic to colleagues at the recently opened veterinary school at Alfort. Anthrax had also been occurring episodically in Europe. However, given the school's receipt of such detailed epidemic reports from the Caribbean colonies, including the clearest documentation to date of the means of cattle-to-human transmission, the Saint-Domingue epizootic/epidemic and related ones must have played a role in the classical first characterization of anthrax by Alfort's director Philibert Chabert in 1780 (3). Chabert's treatise seems to draw directly on the Saint-Domingue reports forwarded by his colleagues, one of whom was Chabert's mentor and the founder of the French veterinary schools, Henri Bertin (10).

Historians have occasionally speculated that large-scale anthrax epidemics occurred in the past (e.g., one of the Pharaonic plagues described in the biblical book of Exodus, occurring around 1491 BC, and an epidemic in seventeenth century Europe [15]), but evidence is weak. Anthrax has also been proposed as the cause of the notorious "Plague of Athens" in 430 BC, a proposition consistent with signs and symptoms of intestinal anthrax in humans and possibly epizootic involvement of dogs and birds of prey (16).

Like the Athenian epidemic—considered by Friedrich Prinzing to be anthrax and included by him among the classic "diseases resulting from wars" (17)—the Saint-Domingue epidemic occurred during a time of upheaval, coming as it did during a devastating earthquake, impending famine, slave revolt, trade wars, and ongoing discord between French and

Spanish colonists. The possibility of biological warfare in either epidemic, however, seems remote. Several years before the 1770 epidemic, during the French-Indian War, British general Lord Jeffrey Amherst wrote a letter in which he discussed giving smallpox-contaminated blankets to North American Indians, and some historians believe the British actually did so (18). By 1770, the French-Indian Wars were over; little would have been gained on any side by harming both French and Spanish colonists, as well as slaves and free residents.

Historical evidence from "natural experiments," such as the 1770 Saint-Domingue epidemic, should be considered in public health efforts to prevent disease re-emergence and increase awareness about bioterrorism potential. In developing countries, single cases and small clusters of severe and fatal intestinal anthrax still occur, often in association with butchering ill animals to obtain consumable and salable meat before the animals die. Such occurrences underscore the importance of efforts to maintain a safe food supply. If historically recorded and widespread intestinal anthrax transmission via broadly distributed meats is accepted as accurate, this 200-year-old evidence would reinforce the need to be vigilant in maintaining safeguards to prevent accidental and purposeful contaminations of food products.

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References

1. Justin M-P. Livre Troisième. In: Justin M-P. Histoire politique et statistique de l' Ile d'Hayti, Saint-Domingue, écrite sur les documents officiels et des notes communiqués par Sir James Barskett. Paris: Brière; 1826. p. 109–25.
2. Meyer J. Introduction. L'enquête de l'Académie de Médecine sur les épidémies 1774-1794. In: Desaive J-P, Goubert J-P, Le Roy Ladurie E, Meyer J, Muller O, Peter J-P, editors. Médecins, climat et épidémies à la fin du XVIII^{e} siècle. Paris: Mouton & C^{ie}; 1972:9–20.
3. Chabert P. Description et traitement du charbon dans les animaux. Paris: Imprimerie royale, 1780.
4. Davaine C. Recherches sur la nature et la constitution anatomique de la pustule maligne. Comptes rendus hebdomadaires des séances de l'Académie des Sciences (Paris) 1865;60:1296–9.
5. Willius FA, Keys TE. The medical history of George Washington (1732–1799). II. Proceedings of Staff Meetings of the Mayo Clinic 1942;17:107–12.
6. Webster N. On the connection of earthquakes with epidemic diseases, and on the succession of epidemics. Medical Repository 1801;4:340–4.
7. Webster N. Historical view of pestilential epidemics from the year 1701, to the year 1788. In: Webster N. A brief history of epidemic and pestilential diseases with the principal phenomena of the physical world, which precede and accompany them and observations deduced from the facts stated. Volume I. Section VII. Hartford (CN): Hudson and Goodwin, 1799. p. 216–82.
8. Webster N. Bills of mortality for the two last centuries, with the principal phenomena of the elements. In: Webster N. A brief history of epidemic and pestilential diseases with the principal phenomena of the physical world, which precede and accompany them and observations deduced from the facts stated. Volume II. Section IX. Hartford (CN): Hudson and Goodwin; 1799. p. 1–9.
9. Katz AR, Morens DM. Severe streptococcal infections in historical perspective. Clin Infect Dis 1992;14:298–307.
10. Bertin H-L-J-B. Relation de quelques accidens extraordinaires, observés à la Guadeloupe, sur les Nègres du quartier de la Capesterre à la suite de l'usage qu'ils ont fait de la chair des animaux morts d'une maladie épizootique. In: de Montigny É-M. Instructions et avis aux habitans des provinces méridionales de la France, sur la maladie putride et pestilentielle qui détruit le bétail. Paris: Imprimerie royale; 1775. p. 49–70.
11. von Heusinger CF. Die Milzbrandkrankheiten der Thiere und des Menschen. Historisch-geographisch-pathologisch Untersuchungen. II. Chronologische Uebersicht der Milzbrandseuchen, welche geherrscht haben. Erlangen: Ferdinand Enke; 1850. p. 93–187.
12. Fleming G. Period from A.D. 1746 to A.D. 1774. In: Fleming G. Animal plagues: history, nature, and prevention. Volume I. Chapter VII. London: Bailliere, Tindall and Cox; 1871. p. 382–464.
13. Law J. Anthrax (malignant pustule). In: Pepper W, Starr L, editors. A system of practical medicine. By American authors. Volume I. Pathology and general diseases. Philadelphia: Lea Brothers and Co.; 1885. p. 926–44.
14. Arthaud C. Épizootie de Saint-Domingue en 1774 & 1775. In: Arthaud C; Cercle des Philadelphes, editors. Recherches, mémoires et observations sur les maladies épizootiques de Saint-Domingue, recueillis & publiés par le Cercle des Philadelphes du Cap-François. Cap-François, Saint-Domingue: L'imprimerie royale; 1788. p. 11–18.
15. Klemm DM, Klemm WR. A history of anthrax. J Am Vet Med Assn 1959;135:458–62.
16. Morens DM, Littman RL. Epidemiology of the plague of Athens. Transactions of the American Philological Association 1992;122:271–304.
17. Prinzing F. The time before the thirty years' war. In: Westergaard H, editor. Epidemics resulting from wars. Oxford: Clarendon Press; 1916.
18. Duffy J. Smallpox and the Indians in the American colonies. Bulletin of the History of Medicine 1951;25:324–41.

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Epidemiologic Response to Anthrax Outbreaks: Field Investigations, 1950–2001

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We used unpublished reports, published manuscripts, and communication with investigators to identify and summarize 49 anthrax-related epidemiologic field investigations conducted by the Centers for Disease Control and Prevention from 1950 to August 2001. Of 41 investigations in which *Bacillus anthracis* caused human or animal disease, 24 were in agricultural settings, 11 in textile mills, and 6 in other settings. Among the other investigations, two focused on building decontamination, one was a response to bioterrorism threats, and five involved other causes. Knowledge gained in these investigations helped guide the public health response to the October 2001 intentional release of *B. anthracis*, especially by addressing the management of anthrax threats, prevention of occupational anthrax, use of antibiotic prophylaxis in exposed persons, use of vaccination, spread of *B. anthracis* spores in aerosols, clinical diagnostic and laboratory confirmation methods, techniques for environmental sampling of exposed surfaces, and methods for decontaminating buildings.

The intentional release of *Bacillus anthracis* in October 2001 greatly challenged the U.S. public health system. Collaborating with partners in other federal, state, and local health agencies, the Centers for Disease Control and Prevention (CDC) responded to these bioterrorism events by relying on experience investigating public health aspects of anthrax over the past 50 years (1). Topics addressed in these investigations included epidemiology, vaccines (2,3), controlling anthrax in industrial and agricultural settings (4), public health response to bioterrorism events (5), *B. anthracis* contamination of milk and meat (6), identifying *B. anthracis*–contaminated commercial products (7), decontamination methods for contaminated environmental sites, and laboratory methods, among others.

Field studies conducted by the Epidemic Intelligence Service (EIS) constituted the cornerstone of these investigative efforts (8). When invited by a state health department or national ministry of health, CDC's EIS Officers conduct field investigations, Epidemic-Aids (known as Epi-Aids), in response to acute public health needs in the United States and other countries. Recently, historic documents from >4,000 Epi-Aids (approximately 90% domestic, 10% international) from 1950 to 1999 were made more accessible through the creation of an internal, searchable electronic database. It includes many unpublished CDC reports on early anthrax investigations, which form the basis of this report.

B. anthracis, the gram-positive, spore-forming, rod-shaped bacterium that causes anthrax (9), is most commonly a zoonotic pathogen. Human *B. anthracis* infections are rare in the United States; the number of cases has decreased steadily from an average of 35 reported cases per year in the 1950s to <1 reported case per year since 1980 (10,11) (Table 1). Most reported cases have been cutaneous. Before October 2001, the last case of inhalational anthrax in the United States occurred in 1976 (12,13).

To answer questions raised when the bioterrorism-related cases of anthrax were identified in October 2001, we reviewed results of field investigations of anthrax. We also identified current questions for which past experience with anthrax provided relatively little information and for which further research is needed.

Methods

CDC anthrax-related field investigations from 1950 to 2001 were identified from several sources. First, the new database of historical Epi-Aid documents (1950–1999) was searched to retrieve all documents in which "anthrax" or "anthracis" appeared either as an assigned keyword or as a text string in a full-text search. Epi-Aid documents related to anthrax investigations in 2000 and 2001 were identified manually in an EIS administrative database. These searches identified a variety of types of documents, including initial requests for epidemiologic assistance, interim progress reports, final reports, and memoranda.

To identify published reports on these Epi-Aid investigations, we searched indexes to the Morbidity and Mortality Weekly Report (MMWR) for anthrax-related reports for the years 1961–2001. The individual issues of MMWR and its predecessor (Weekly Morbidity Report) were searched manually for the years (1950–1960) for which no index exists. To identify published reports on anthrax-related Epi-Aid investigations, we searched Medline for the years 1966–2001 and

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Table 1. CDC field investigations of suspected anthrax in humans and animals, and reported cases of anthrax in humans, United States, 1950–2001[a]

Years	Field investigations				No. of cases of anthrax in humans reported nationally[c]
	No. of investigations[b]	No. of human cases			
		Cutaneous	Inhalational	Total	
1950–54	2	1	0	1	223
1955–59	11	16	6	22	131
1960–64	4	5	1	6	54
1965–69	7	5	1	6	21
1970–74	8	4	0	4	13
1975–79	6	5	1	6	10
1980–84	0	0	0	0	2
1985–89	1	1	0	1	3
1990–94	1	0	0	0	1
1995–99	2	0	0	0	0
2000–01[d]	2	2	0	2	Not available
Total	44	39	9	48	458

[a]CDC, Centers for Disease Control and Prevention.
[b]Excludes three investigations of suspected anthrax conducted outside the United States (1967, 1986, 1998) and two investigations focused on decontamination of *Bacillus anthracis*–contaminated textile mills (1967, 1972).
[c]Sources: CDC. MMWR Summary of Notifiable Diseases, United States, 1994 (10); and MMWR Summary of Notifiable Diseases, United States, 1999 (11).
[d]Before October 2001 bioterrorism-related anthrax cases.

Index Medicus for 1950–1965. The names of the lead investigators from the Epi-Aids were used as keywords.

Additional CDC anthrax-related field investigations were identified by two coauthors (PB and AK) who were personally involved in most anthrax investigations conducted by the agency since the 1950s. References describing these additional investigations were located in the MMWR and in published medical articles. To limit this report to a description of CDC's institutional experience, rather than a broader review of publications on anthrax investigations, we excluded (a) anthrax case reports published in the MMWR but unrelated to a CDC field investigation and (b) published reports on anthrax by investigators not affiliated with CDC.

From the unpublished Epi-Aid documents and published reports for each investigation, we abstracted the following information: year, location, number of human and animal cases, clinical form of the disease, occupational or other exposures for human patients, environmental sampling methods and data, and study recommendations.

Results

A total of 49 relevant field investigations (Table 2) were included in this report: 42 Epi-Aids and 7 other investigations. Detailed reports and MMWR published summaries were available for 39 (93%) of the 42 Epi-Aid field investigations. For three agriculture-related investigations (Epi-Aids 1963-2, 1959-38, 1957-17), only the initial invitation for epidemiologic assistance was available for review.

Of these 49 field investigations, 41 (84%) involved human or animal infections with *B. anthracis*, 2 were evaluations of decontamination of *B. anthracis*–contaminated textile mills (1967 and 1972), and 1 was an investigation of bioterrorism threats involving anthrax (1998). In the other 5 investigations, *B. anthracis* was not found to be the causative organism, despite initial suspicion. Because these investigations include only anthrax cases for which CDC's assistance was requested, they represent only a small proportion of the total number of U.S. cases reported during this period (Table 1).

Most of the investigations (41/49, 84%) were conducted from 1950 to 1980; only 8 anthrax-related investigations were conducted by CDC from 1980 until the October 2001 bioterrorism events. This trend mirrors the decline in reported U.S. anthrax cases in the latter half of the 20th century (10) (Table 1).

Site

Among the 41 field investigations involving infection with *B. anthracis* (Table 2), 24 involved an agricultural setting (farms, contact with livestock, or both), 11 textile mills, 4 *B. anthracis*–contaminated commercial products, and 1 contaminated cow bones; in 1 instance, the source of infection was not determined. Thirty-eight (93%) of the 41 investigations took place in the United States; other investigations were conducted in Haiti (1974), Paraguay (1986), and Kazakhstan (1998).

Clinical Form and Mechanism of Infection

All U.S. investigations involved cutaneous or inhalational anthrax (Table 3). Excluding large outbreaks in Kazakhstan and Paraguay, investigations in this report include 39 cutaneous and 9 inhalational cases of human anthrax. Among the investigations with available information on age and sex of

patients, ages ranged from 19 to 67 years (median 40 years), and most cases were in males (Table 3).

Routes of infection were largely a function of setting. Of the 27 cases in textile mills, 21 (78%) were cutaneous, and 6 (22%) were inhalational. Contaminated goat hair or wool was the primary vehicle of infection. Persons working with raw, unprocessed materials were at greatest risk for infection (4). Of the six inhalational cases in textile mills, five were fatal. Three cases of fatal inhalational anthrax were also reported in non-textile mill workers (12,31) (Epi-Aid 1967-43).

Of the 24 investigations in agricultural settings, 9 (38%) included at least one human case. All human cases were acquired cutaneously while a person was handling, performing necropsy on, or disposing of dead animals. The most extensive cross-infection between animals and humans occurred in the 1998 outbreak in Kazakhstan, in which at least 53 human cases occurred; most were cutaneous cases acquired from slaughtering animals (Epi-Aid 1998-83).

Although four investigation reports included concern over possible waterborne transmission ([15] and Epi-Aids 1966-12, 1975-6, 1979-95), this route was not identified in any of the reports of human cases, and water contamination was not regarded as a source of infection. However, disease incidence in animals usually coincided with extremes of wet and dry weather conditions.

Gastrointestinal anthrax was documented in one investigation. Of the 53 persons with anthrax in the 1998 Kazakhstan outbreak, 2 were diagnosed with gastrointestinal anthrax after eating contaminated raw meat. In 1968 in Connecticut, 204 kg of *B. anthracis*–contaminated meat was sold as hamburger before the epizootic investigation; although purchasers of the meat could not be located, no human cases of anthrax were known to have occurred from the contaminated meat (Epi-Aid 1968-78). In addition, in 2000, a Minnesota farm family ate well-cooked meat from a *B. anthracis*–infected steer. Some family members had gastrointestinal symptoms, but investigators could not confirm or rule out infection with *B. anthracis* (54).

Human Prophylaxis

In nine outbreaks, 136 persons were documented to have received antibiotic postexposure prophylaxis. In at least five of the investigations, postexposure prophylactic therapy was stopped once additional information about risk became available. None of these reports described subsequent infections in patients who received any prophylaxis. Early prophylactic regimens used penicillin injections, which were later replaced by tetracycline, then doxycycline and quinolones, administered orally or parenterally (Epi-Aids 1966-18, 1999-25). Prophylactic antibiotics have been recommended in specific cases involving direct physical contact with contaminated material, but are not routinely recommended because the risk for an adverse drug reaction may exceed the risk for infection (Epi-Aid 1975-6). In one report describing a series of events not consistent with public health recommendations, a worker who was potentially exposed to *B. anthracis* in a rendering plant placed a large bottle of tetracycline on a lunchroom table, and coworkers took various amounts of antibiotics if concerned about potential exposure (Epi-Aid 1979-95).

In a 1962 field investigation, an acellular anthrax vaccine was demonstrated to be 93% effective in reducing the risk for infection with *B. anthracis* in humans. The vaccine was subsequently recommended for persons who handle imported hair, wool, hides, or bone meal (2).

Occupational Exposures

In 23 of the 27 U.S. investigations involving human anthrax, exposures occurred in occupational settings. The other four investigations involved exposure to contaminated commercial products or to aerosolized *B. anthracis* spores while a person was passing close to contaminated industrial mills. Among persons exposed in textile mills, most affected workers had direct contact with wool and goat hair as part of their job. However, in 1961, fatal inhalational anthrax occurred in a secretary at a goat hair–processing mill (Epi-Aid 1961-40), and in 1966, cutaneous anthrax occurred in a truck driver who helped unload baled goat hair at a mill (Epi-Aid 1967-43).

In agricultural settings, most cases were in ranchers or other workers who were exposed during the slaughter, butchering, or disposal of *B. anthracis*–infected animals. During 1957–1971, cutaneous anthrax occurred in six veterinarians after they performed necropsies on infected animals; one veterinarian had not used gloves during the necropsy, another had an anthrax lesion on his wrist (suggesting it was uncovered), and no information is available about glove use by the other veterinarians. Other occupational exposures include the goat hair exposures of a pipe insulator in Ohio (51,52) and a weaver in California (12).

Environmental and Clinical Testing

Specific environmental sampling methods were described in 26 (59%) of the 44 investigations. Sampling methods varied by setting. In textile mills, investigators usually tested samples from raw and processed materials, especially goat hair and wool. In nine investigations, air and surface samples were also tested from numerous locations in and around the mills. In 1978 in North Carolina (Epi-Aid 1978-47), 300 soil samples were taken from the mill premises, the landfill, and private residences near the mill; none tested positive for *B. anthracis*. Samples were also tested from floor sweepings and vacuum cleaner contents from inside the homes of four mill workers; one sample tested positive for *B. anthracis*. In 1953 in North Carolina (Epi-Aid 1953-14), two guinea pigs and four mice were exposed to the air near operating machines in the mill for 3½ hours; no test results are available. No reports of the subsequent investigations of textile mills mentioned the use of such animal tests for environmental sampling during an acute epidemic, although primates were experimentally exposed to air from a *B. anthracis*–contaminated textile mill in South Carolina (55).

Table 2. Characteristics of CDC field investigations of anthrax in humans and animals, 1950–August 2001[a]

Year	Location	No. of cases		Reference	Comments
		Human	Animal		
Agricultural settings (n=24 investigations)					
2001	TX (southwest)	1	1,638	Epi-Aid 2001-61	Large epizootic affecting 63 properties in five counties; members of at least 11 animal species were infected with *Bacillus anthracis.*
2000	ND (east)	1	Multiple	Epi-Aid 2000-69, (14)	USDA recommended quarantine on affected premises, vaccinating livestock on surrounding premises, and burning and/or burying infected carcasses, bedding, and other nearby materials.
1998	Kazakhstan	At least 53	Multiple	Epi-Aid 1998-83	Multivariate analysis found highest risk for cutaneous anthrax from slaughtering, butchering, and cutting *B. anthracis*–infected animals; eating cooked infected meat not an important risk factor.
1998	Uvalde, TX	One vaccine exposure	0	Epi-Aid 1998-55	Patient accidentally exposed to attenuated live anthrax vaccine while vaccinating horse, experienced severe myalgia and fatigue, then began antibiotic prophylaxis and recovered. Laboratory tests negative for *B. anthracis*.
1993	ND (southeast)	0	8	(15)	NIOSH and USDA investigation following major flooding, anthrax in livestock, and soil contamination. Concern over contaminated water supply, but all water samples negative.
1986	Paraguay	At least 21	0	Epi-Aid 1986-39, (16)	Community outbreak of cutaneous anthrax in a remote village.
1979	Clay County, IA	0	16	Epi-Aid 1979-95	Raising chlorine level to 2 ppm eliminated two positive samples in well water. In local hospital records, no difference in number of gastrointestinal symptoms compared with same month in previous year.
1976	Foard and Cottle Counties, TX	0	≥ 160	Epi-Aid 1976-115, (17)	Significantly higher attack rates in bulls and horses; evidence against flies as important vector.
1974	Falls County, TX	0	≥ 236	Epi-Aid 1975-6, (18,19)	*B. anthracis*– positive sample from city water tap, so city water supply was hyperchlorinated. Soil samples collected to document efficacy of carcass incineration were negative.
1971	Danville, PA	0	33	Epi-Aid 1972-19	*B. anthracis* isolated from both hay and soil samples.
1971	Gonzales, LA	2	588	Epi-Aid 1971-131, (3,20,21)	One culture positive and one negative in exposed veterinarians. Low attack rate in calves reduced likelihood that biting flies were an important vector.
1970	Yoder, WY	0	8	Epi-Aid 1971-44, (22)	Veterinarian placed on antibiotic prophylaxis as a result of laceration while performing necropsy.
1968	Inyo County, CA	1	176	Epi-Aid 1969-20, (23)	Extensive discussion and literature review of *Tabanid* species (horsefly) as potential vector; role in transmission remains inconclusive.
1968	Hampton, CT	0	3	Epi-Aid 1968-78	204 kg of *B. anthracis*–contaminated meat sold as hamburger before investigation. No human cases of anthrax known to have occurred as a result.
1965	Grand Forks, ND	0	19	Epi-Aid 1966-12, (24)	30 diabetic children swam 3 miles downstream from where an animal was found dead from anthrax; riverborne spread determined minimal; prophylaxis considered unnecessary.
1962	MS	0	Multiple	Epi-Aid 1963-2	Involved many counties.
1959	Brownsville, Cameron County, TX	5	125	Epi-Aid 1960-12	Two cases laboratory confirmed. Cases occurred in three veterinarians and two other patients who had intimate contact during necropsy, handling, or skinning.
1959	NJ (south)	1	2 cows, many hogs	Epi-Aid 1959-38	Not laboratory confirmed. Several hogs developed illness after feeding on entrails of sick cows.
1958	LA (north)	0	15–20	Epi-Aid 1958-42	Involved cows, sheep, and horses.
1957	Vinita, OK	1	400–500	Epi-Aid 1958-11, (25)	Large epizootic on farms curtailed after intensive immunization campaign.
1956	Saratoga, WY	0	Multiple	Epi-Aid 1957-17	Animal anthrax in mountainous area led to concern over water supply downstream.

Table 2 continued. Characteristics of CDC field investigations of anthrax in humans and animals, 1950–August 2001[a]

Year	Location	No. of cases		Reference	Comments
		Human	Animal		
1956	MS (northwest)	0	>250	Epi-Aid 1957-3	No evidence to support insectborne transmission, despite local beliefs. Involved 224 head of cattle, 42 mules, 5 horses, 3 sheep, 2 goats, multiple hogs. One case of suspected anthrax in a child was investigated and determined to be mumps.
1955	LA (southeast)	0	1,404	Epi-Aid 1955-5	Large epizootic in cattle. Unconfirmed reports of four human cases. *B. anthracis* isolated from flies in two instances at State Animal Disease Laboratory.
1952	OH (five counties)	0	Multiple	Epi-Aid 1952-13, (26)	*B. anthracis* isolated from swine feed; contaminated bonemeal suspected as source of infections.
Textile mills (n=13 investigations)					
1987	Charlotte, NC	1	0	Epi-Aid 1987-77, (27)	Suspected cross-contamination of Australian wool from storage space shared with contaminated West Asian cashmere.
1978	NH (southeast)	2	0	Epi-Aid 1978-65	Patients did not wear protective equipment. One had systemic signs and symptoms (fever, headache, sore neck, malaise, anorexia) after his initial lesion was lanced. Subsequent full recovery.
1978	Shelby, NC	2	0	Epi-Aid 1978-47	Contents of vacuum cleaner bags or floor sweepings from four employee homes were collected; 1 tested positive for *B. anthracis*. 300 soil samples tested from mill premises, landfill site, and nearby residences. In mill, more positive samples in rooms where earliest processing occurred.
1974	Belton, SC	1	0	Epi-Aid 1974-77	Report suggested prevention should be based on minimizing contact between employees and contaminated material, and on routine vaccination of employees at risk. Patient not adequately vaccinated.
1972	Manchester, NH	N/A	N/A	Epi-Aid 1972-94	Effectiveness of formaldehyde vapor decontamination of *B. anthracis* spores assessed using spore strips in treated and untreated (control) areas of mill complex, and comparing pre- and posttreatment surface samples. No positives among 599 posttreatment specimens.
1967	Dillon, SC	N/A	N/A	(28)	A building contaminated with *B. anthracis* was successfully decontaminated with formaldehyde vapor. 100,000 spores on 24 plates pretreatment were reduced to 21 sterile plates, and 3 plates with 2 colonies each, posttreatment. 26 of 142 surface swabs tested positive before decontamination, and 1 of 200 swabs tested positive 6 months after decontamination. Building was deemed safe for occupancy and no further cases were reported.
1966	Manchester, NH	2	0	Epi-Aid 1967-43	Patient with inhalational anthrax had history of "smoker's cough," diabetes, alcoholism, and chronic pancreatitis. Exposure believed to have occurred while patient worked for 4–5 hours directly opposite a goat hair–processing mill.
1961	Philadelphia, PA	1	0	Epi-Aid 1961-40; (29)	After case reported, supplies of new and improved Wright vaccine sent to mill for use among employees.
1960	SC	4	0	Epi-Aid 1960-31, (30)	All four cases responded well to antibiotic treatment.
1957	Philadelphia, PA	1	0	(31,32)	Two additional inhalational cases mentioned that occurred over an 8-year period in persons living near the same contaminated tannery.
1957	Manchester, NH	9	0	Epi-Aid 1958-18, (33–36)	Employees noted increased dust in air after initiating a new scouring technique in textile mill.
1956	Monroe, NC	≥5	0	Epi-Aid 1956-29, (37)	Studies indicated heavy environmental contamination of mill with *B. anthracis* spores.
1953	Monroe, NC	1	0	Epi-Aid 1953-14	Nasal swabs of employees performed to assess exposure. No results available.
Other settings (n=7 investigations)					
1998	CA, IN, KY, TN	0	0	Epi-Aid 1999-25, (38)	Evaluation of multiple telephone threats and letters alleged to contain *B. anthracis*. Report included recommendations for response to bioterrorism threats.

Table 2 continued. Characteristics of CDC field investigations of anthrax in humans and animals, 1950–August 2001[a]

Year	Location	No. of cases		Reference	Comments
		Human	Animal		
1976	Morro Bay, CA	1	0	(12,39,40)	Suspected source of anthrax in home craftsman was contaminated yarn imported from Pakistan. Multiple samples of yarn tested positive for *B. anthracis*. Subsequent CPSC warning on imported yarn.
1975	Camden, NJ	3	0	(41–43)	Cutaneous anthrax in three gelatin manufacturing plant workers from contact with contaminated dry cattle bones; FDA recall of dicalcium phosphate animal feed product.
1974	Sequim, WA	0	42	(44,45)	Several cougars and other large felines on private game farm died after feeding on infected horsemeat. Primary source: horse's saddle pad contained *B. anthracis*–contaminated goat hair from Afghanistan and Pakistan. Subsequent CPSC warning on contaminated saddle pads.
1974	Haiti; FL	1 in US; 194 in Haiti (1963-1974)	0	Epi-Aid 1974-96, (7,46–50)	One human case in U.S.; 194 cases identified in Haiti in 1963–1974. 72 (25%) of 287 Haitian goatskin handicrafts tested from January to May 1974 were culture positive for *B. anthracis*, including voodoo balancing dolls, rugs, whole skins, mosaic pictures, purses, and drums. Subsequent CPSC warning on contaminated Haitian goatskin products.
1966	Manchester, NH	1	0	Epi-Aid 1967-43-3	Source of cutaneous infection in housewife unknown, but knitting yarn could not be ruled out. Three samples from knitted sweater positive for *B. anthracis*; samples from other sources negative.
1964	Oxford, OH	1	0	(51,52)	Fatal cutaneous anthrax in installer of pipe insulation made with imported goat hair. Insulation and goat hair samples tested positive for *B. anthracis*.
Suspected anthrax shown due to other causes (n=5 investigations)					
1975	Yavapai County, AZ	1	0	Epi-Aid 1975-115	23-year-old male machinist initially thought to have anthrax but quickly determined to have plague.
1969	Casper, WY	1	0	Epi-Aid 1969-78	Meat packing company employee; anthrax thought not to be responsible.
1967	Nepal	26	Multiple	Epi-Aid 1968-34	Community outbreak of cutaneous disease; subsequently diagnosed as plague.
1965	Charleston, SC	1	0	Epi-Aid 1966-18, (53)	Cutaneous disease in customs inspector; *B. anthracis* not implicated.
1957	Jamestown, NY	5	0	Epi-Aid 1958-16	Cutaneous disease in butchers; later believed to be a streptococcal or staphylococcal infection.

[a]CDC, Centers for Disease Control and Prevention; CPSC, Consumer Product Safety Commission; OSHA, Occupational Safety and Health Administration; FDA, U.S. Food and Drug Administration; USDA, U.S. Department of Agriculture; NIOSH, National Institute for Occupational Safety and Health.

In agricultural settings, investigators frequently tested samples of soil, water, and animal carcasses. Environmental sampling was specifically mentioned in 13 agricultural investigations. Elaborate systematic sampling strategies for soil were sometimes used, such as in Louisiana in 1971 (Epi-Aid 1971-131) and in Texas in 1974 (Epi-Aid 1975-6). In other investigations, objects that tested positive for *B. anthracis* in farm settings included hay in Pennsylvania in 1971 (Epi-Aid 1972-19), biting flies in Louisiana in 1955 (Epi-Aid 1955-5), and swine feed made from *B. anthracis*–contaminated bonemeal in Ohio in 1952 (Epi-Aid 1952-13).

During a series of anthrax threats and hoaxes in 1998 (38) (Epi-Aid 1999-25), samples from mailed letters were tested for *B. anthracis* spores by phase microscopy in a university microbiology laboratory, cultured for *B. anthracis* in Laboratory Response Network Level B laboratories (56), and subjected to rapid antigen testing by the U.S. Army Medical Research Institute for Infectious Diseases. All samples from letters were negative. Environmental samples taken from buildings after telephoned threats of contaminated air-handling systems were also negative. In other investigations, objects tested for *B. anthracis* were goat hair pipe insulation (52), imported yarn (12), a knitted sweater (Epi-Aid 1967-43-3), goat hair from contaminated horse saddle pads (44), and Haitian goatskin handicrafts at various stages of the manufacturing process (46,47) (Epi-Aid 1974-96).

With regard to clinical testing in human cases, most detailed reports mention smears and cultures being done on skin lesions and blood samples. Some of these tests were conducted after antibiotics had been started, thereby reducing the likelihood of a positive result. Several of the more recent investigations included serologic tests for antibodies to *B. anthracis* antigens but did not assess the utility of these clinical assays. Nasal swabs were collected from 37 workers during a 1953 North Carolina textile mill anthrax investigation (Epi-Aid 1953-14); laboratory results are not available. No

other investigations mentioned use of nasal swabs, and the effectiveness of nasal swabs in detecting *B. anthracis* infection was not discussed in the reports reviewed.

Decontamination

Several reports recommended specific measures for decontaminating affected areas or materials. A 1953 report suggested that all dirt, dust, and sweepings from a potentially contaminated textile mill be burned (Epi-Aid 1953-14). A 1960 report indicated that a livestock rendering plant was "cleaned up in the recommended manner with 5% hot lye solution" (Epi-Aid 1960-12). A 1967 report recommended installation of a high-temperature furnace at the textile mill for burning wastes (Epi-Aid 1967-43). A 1978 report recommended that potentially contaminated textile mill wastes be soaked in a 5% formaldehyde solution before burial in a landfill (Epi-Aid 1978-65).

The report on Epi-Aid 1972-94 contains the most detail on building-decontamination procedures. In this investigation, an unoccupied New Hampshire textile mill complex slated for demolition was decontaminated. Recommendations were based in part on experience in the earlier decontamination of two South Carolina mill buildings (28); those buildings were subsequently used by another industry for >2 years without any cases of human anthrax being reported. The New Hampshire mill buildings were decontaminated with 9,691 L of liquid formaldehyde that was vaporized and delivered into the interior rooms of the sealed buildings. None of 260 spore strips containing *B. anthracis*, *B. globigii* (now known as *B. atrophaeus),* or *B. subtilis* placed in treated areas of the mill complex showed growth; 23 of 40 such strips placed in untreated (control) areas showed spore growth. In addition, 2 of 555 surface swabs tested positive before treatment, but none of 599 swabs tested positive after treatment. These data from spore strips and surface swabs suggest that the decontamination process was effective in reducing and possibly eliminating the environmental contamination with *B. anthracis*.

During a 1974 anthrax epizootic in Texas (Epi-Aid 1975-6), investigators evaluated the disposal of infected animal carcasses by burning them with old tires, wood, and crank case oil. All 21 samples of carcass ashes, underlying soil, and soil up to 1 m from the burn site were negative for *B. anthracis*.

Cross-Contamination

Two reports mentioned evidence of cross-contamination from a primary contaminated object to another object or site. In a North Carolina textile mill in 1987 (Epi-Aid 1987-77), investigators speculated that the sample of *B. anthracis*–contaminated Australian wool had been cross-contaminated by *B. anthracis*–contaminated West Asian cashmere stored in the same room. During another North Carolina anthrax outbreak in 1978 (Epi-Aid 1978-47), one of four vacuum cleaner dust samples from the homes of textile mill workers was positive for *B. anthracis*, suggesting that workers carried spores on their clothes from the mills to their homes. No cases of anthrax in workers' families were reported, suggesting that exposures to *B. anthracis* in the home were not clinically significant.

Misidentification of Cutaneous Anthrax

A complete differential diagnosis of the clinical manifestations of anthrax includes many other diseases (57,58). In five investigation reports and one MMWR case report, cutaneous lesions initially diagnosed as possible anthrax were subsequently attributed to other diseases (Table 2). In 1975, anthrax was initially suspected in a 23-year-old Arizona man, but his illness was quickly determined to be plague (Epi-Aid 1975-115). In 1973, two sisters in California developed vesiculopapular lesions on their fingers after contact with ill lambs. Anthrax was suspected, but the cultures were negative, and the disease was diagnosed as human orf (59). In 1969, investigators determined that a gram-positive spore-forming bacillus from a skin lesion on a Wyoming meat-packing company worker was not *B. anthracis*, but no definitive species identification could be made (Epi-Aid 1969-78). *B. anthracis* was initially suspected as the cause of cutaneous lesions in persons in a remote village in Nepal in 1967, but plague was subsequently documented (Epi-Aid 1968-34). In 1965, laboratory samples from a skin lesion of a South Carolina customs inspector who had had contact with imported wool were negative for *B. anthracis*. Although no definitive diagnosis was made, the clinical picture made anthrax unlikely (Epi-Aid 1966-18). Finally, in 1957, cutaneous lesions on five New York butchers initially considered as possible anthrax were subsequently diagnosed as pyoderma caused by staphylococci, streptococci, or both (Epi-Aid 1958-16).

Recommendations and Impact of Investigations

Field investigation reports usually contain public health recommendations; many of these are appropriate for future anthrax epidemics or exposures. For infections associated with textile mills, a 1974 report stated that "decontamination of the primary source of *B. anthracis* is not generally held to be practical" (Epi-Aid 1974-77). The reports on textile mill investigations recommended anthrax vaccine with annually scheduled booster inoculations for mill workers at risk; use of personal protective equipment including specific work clothing and respirators, shower facilities, and separate lockers for work and street clothing; physical separation of raw and finished materials to prevent cross-contamination; design of work areas for easy cleaning; and air-exhaust systems designed to prevent the spread of spores. One report recommended that mill employees be "thoroughly indoctrinated" on the cause, nature, and control of anthrax (Epi-Aid 1953-14). In 1999, following multiple bioterrorist threats (38) (Epi-Aid 1999-25), antibiotic prophylaxis was recommended in cases with known or credible risk for direct exposure. For persons with suspected exposure to aerosolized spores, recommendations included isolating exposed clothing in a plastic bag, showering with copious amounts of soap and water, and washing all possibly contaminated materials with a 1:10 bleach dilution (38).

Table 3. Inhalational and cutaneous anthrax in humans in CDC field investigations, United States, 1950–2001[a]

Year	Location	Occupation	Source	Age, sex	Reference[b]	Comments
Inhalational (n=9 cases)						
1976	Morro Bay, CA	Self-employed weaver	Imported yarn	32, M	(12)	Fatal inhalational anthrax due to contaminated imported yarn containing goat hair.
1966	Manchester, NH	Metal shop employee	Nearby mill processing goat hair	46, M	Epi-Aid 1967-43	Dust from neighboring goat hair mill identified as source. Incidence of anthrax at plant decreased with mandatory vaccination. Patient's coexisting illnesses may have contributed to susceptibility.
1961	Philadelphia, PA	Secretary in textile mill	Goat hair	50, F	Epi-Aid 1961-40	Fatal inhalational anthrax. Unusual because little contact with goat hair in routine work duties.
1957	Manchester, NH	Gillboxer in textile mill	Goat hair	60, M	Epi-Aid 1958-18	Five inhalational cases of anthrax (four fatal) occurred in the 600 employees of a textile mill. Four cutaneous cases occurred during the same outbreak.
		Bobbin cleaner and weaver		65, F		
		Card fixer		49, M		
		Card tender		61, M (recovered)		
		Noil remover		33, M		
1957	Philadelphia, PA	Factory employee	Nearby mill processing goat hair	29, M	(31,32)	Fatal inhalational anthrax in man with sarcoidosis. Possible exposures from glue made from animal hides, or goatskin tannery with sweepings and surfaces testing positive for *Bacillus anthracis*, which patient walked by daily.
Cutaneous[c] (n=39 cases)						
2001	TX (southwest)	Farm worker	Infected animal	?, M	Epi-Aid 2001-61	Exposure during disposal of infected carcasses.
2000	ND (east)	Farm worker	Infected animal	67, M	Epi-Aid 2000-69	Exposure during disposal of infected carcasses.
1987	Charlotte, NC	Maintenance employee	West Asian cashmere	42, M	Epi-Aid 1987-77	Worked in a goat hair–processing mill.
1978	NH (southeast)	Worker at goat hair–processing mill	Goat hair	20, M	Epi-Aid 1978-65	Loaded hair-carding machine and performed other tasks.
				19, M		Worked at hair mixing and carding machines during week before onset of symptoms.
1978	Shelby, NC	Maintenance worker at goat hair–processing mill	Goat hair	59, M	Epi-Aid 1978-47	
		Temporary worker at goat hair–processing mill	Goat hair	67, M		
1974	Belton, SC	Employee at textile mill	Goat hair	38, F	Epi-Aid 1974-77	Worked in mill spinning area.
1974	Haiti; FL	Navy journalist-photographer	Goatskin in Haitian handicrafts	22, F	Epi-Aid 1974-96	Cutaneous anthrax in FL resident after purchase of *B. anthracis*–contaminated goatskin drums in Haiti.
1971	Gonzales, LA	Two veterinarians	Infected cow	52, M; 26, M	Epi-Aid 1971-131	Disease contracted during necropsy.
1968	Inyo County, CA	Farmhand	Unknown	63, M	Epi-Aid 1969-20	Suspected human cutaneous case, in region of horsefly bite; patient responsible for burning cattle carcasses. Cattle and horsefly exposures considered.
1966	Manchester, NH	Truck driver	Goat hair	35, M	Epi-Aid 1967-43	Truck driver helped unload delivered bales despite being instructed not to help.

Table 3 continued. Inhalational and cutaneous anthrax in humans in CDC field investigations, United States, 1950–2001[a]

Year	Location	Occupation	Source	Age, sex	Reference[b]	Comments
1966	Manchester, NH	Unknown	Not determined	35, F	Epi-Aid 1967-43-3	Source uncertain; three samples from hand-knitted sweater positive for *B. anthracis*.
1965, 1969, 1975	Camden, NJ	Three gelatin manufacturing plant workers	Contaminated dry cow bones, used in manufacturing process	29, M; 45, M; ?, M	(41–43)	OSHA fined gelatin factory owners for failure to protect workers.
1964	Oxford, OH	Pipe insulation installer	Goat hair in pipe insulation	36, M	(51)	Fatal cutaneous case featured in a 1965 New Yorker article by Berton Roueche (52).
1960	SC	Four textile mill employees	Goat hair	?	Epi-Aid 1960-31	
1959	Brownsville, Cameron County, TX	Three veterinarians	Necropsy, livestock exposure	?, M; ?, M; ?, M	Epi-Aid 1960-12	One veterinarian had performed necropsy on a steer; other exposures not specified.
		Employee at rendering plant	Not specified	?, M		
		Unspecified	Infected steer	"adolescent boy"		Suspected exposure while skinning steer in Mexico.
1959	NJ (south)	Farmer	Undetermined	23, M	Epi-Aid 1959-38	Possible sources included cows that died of anthrax, and fertilizer with contaminated goat hair.
1957	Vinita, OK	Veterinarian	Infected cow	?, M	Epi-Aid 1958-11	Had performed necropsy on a cow.
1957	Manchester, NH	Two weavers and two card tenders at textile mill	Goat hair	50, F; 64, F; 35, M; 61, M	Epi-Aid 1958-18	
1956	Monroe, NC	Five textile mill employees	Goat hair	?	Epi-Aid 1956-29	
1953	Monroe, NC	Textile mill employee	Goat hair	36, F	Epi-Aid 1953-14	

[a]CDC, Centers for Disease Control and Prevention; OSHA, Occupational Safety and Health Administration.
[b]See Table 2 for additional references.
[c]Excludes investigations in Paraguay and Kazakhstan, where the number of human cases is uncertain.

For infections associated with farms and livestock, reports recommended vaccination of animals at risk, better education of farm workers on anthrax diagnosis and control, thorough destruction by burning of infected animals, prevention of infected livestock from reaching the market, improved supervision of slaughter and meat inspection, and, in some situations, farm quarantine. After the 1974 Texas epizootic (Epi-Aid 1975-06), anthrax vaccine was tested in dairy cattle to assure that the vaccine had no adverse effect on milk safety (6).

Investigations of *B. anthracis*–contaminated saddle pads (1974), Haitian handicrafts (1974), and imported yarn (1976) led to Consumer Product Safety Commission recommendations for destroying those products (7,39,45). In 1975, cutaneous anthrax developed in a New Jersey gelatin manufacturing plant worker after his exposure to contaminated dry cattle bones; the Occupational Safety and Health Administration levied fines for workplace safety violations (41).

The Haitian investigation also led to a federal ban on importing Haitian goatskin products. A review of such handicrafts collected at U.S. quarantine stations in 1980–1981 found that items continued to be contaminated with *B. anthracis* (47). Recommendations to the Haitian Ministry of Health included providing incentives for reporting diseased animals, improving laboratory diagnostic capacity, increasing anthrax vaccination levels among livestock, educating livestock owners about the benefits of anthrax control, and improving the tanning procedures for goatskin drum heads (Epi-Aid 1974-96).

Discussion

In this report we review what has been learned from >40 epidemiologic field investigations of confirmed or suspected anthrax outbreaks in humans or animals during the last 50 years. In the 2001 bioterrorism response, investigators evaluated suspected anthrax cases by using clinical and laboratory diagnostic methods, such as chest radiographs, cultures, and serologic assays, that had been developed and refined during earlier investigations of inhalational and cutaneous anthrax in textile mill workers. In addition, histopathologic and immunohistochemical testing proved essential for diagnosing anthrax in persons who had been placed on antibiotics early and whose cultures were thus negative. Nasal swabs, as used in the 1953 textile mill investigation, are currently considered an unevalu-

ated adjunct to environmental sampling for defining exposed populations in bioterrorism investigations (1,60). Nasal swabs were used in the 2001 investigation for defining the aerosol spread of *B. anthracis* spores in the Hart Senate Office Building and some other settings.

In the 2001 bioterrorism investigation, an anti-protective antigen, enzyme-linked immunosorbent assay (61) was used to confirm *B. anthracis* infection in several cases. Development of this assay was the culmination of decades of laboratory experience and research associated with past field investigations of anthrax.

Asymptomatic infection was documented in one serologic survey (33) conducted several months after an inhalational anthrax outbreak; however, in past and current investigations, the role of asymptomatic infection in providing protection is unclear. Human-to-human spread was not evident in any of the investigations reviewed.

Investigation into a series of anthrax-related threats and hoaxes in 1998 (Epi-Aid 1999-25) also helped lay the groundwork for the recent response. In that investigation, guidelines for risk assessment and postexposure antibiotic prophylaxis were developed, and coordination with first responders and law enforcement was emphasized (38). The investigation also led to revised immunization recommendations (5), which discuss the use of vaccine for postexposure prophylaxis.

In response to the bioterrorism events of 2001, additional guidelines were published on investigating and responding to *B. anthracis* exposures. These address clinical testing, use of antibiotic prophylaxis, closing of potentially contaminated buildings, and postexposure treatment options (1,62,63). Current recommendations for the use of anthrax vaccine are based in large part on a field trial conducted in 1962 (2,5). During the 2001 response, vaccination recommendations were expanded to at-risk populations; the 1962 vaccine efficacy study forms part of the justification for considering the vaccine for postexposure prophylaxis. Currently, the Advisory Committee on Immunization Practices recommends that vaccine be used in combination with antibiotics (ciprofloxacin, doxycycline, or penicillin) following a *B. anthracis* bioterrorism exposure, if vaccine is available (5). Vaccination is a critical component of the nation's preparedness and response activities for *B. anthracis* bioterrorism.

In past field investigations, the primary risk factor for human cutaneous anthrax has been direct physical contact with infected animals or commercial products containing *B. anthracis* spores. Ranchers, butchers, and veterinarians were at risk for such contact when working with infected animals. All the commercial products causing human infection were of animal origin; most were made from imported goat skin or hair.

For inhalational anthrax, the main risk factor was exposure to aerosolized spores, especially in or near a textile mill that processes goat hair. While it is unclear why some workers become infected while others in the same dusty environment do not, several factors may increase the likelihood for infection. First, direct work with unprocessed goat hair may create a heavier exposure to *B. anthracis* spores. Second, a weakened immune system may increase a person's susceptibility to infection (64). Two of the patients with inhalational anthrax probably had chronic pulmonary disease. In the 1957 investigation, sarcoidosis was present (31). In the 1966 investigation of a metal shop worker (Epi-Aid 1967-43), investigators noted the worker's "chronic cigarette cough" and suggested that his alcoholism, diabetes, and pancreatitis might have made him more susceptible than his healthy coworkers.

Over the past 50 years, a series of recommendations have focused mainly on preventing occupationally acquired anthrax, especially in textile mills and agricultural settings. For example, in 1962, anthrax vaccine was recommended for persons who handle imported hair, wool, hides, or bonemeal (2). More recently, it was recommended that veterinarians obtain diagnostic specimens but not perform necropsies on animals suspected to have died from anthrax (36). The National Institute for Occupational Safety and Health has been actively involved in many recent anthrax-related investigations (15,65).

Some documents mentioned insects as possible vectors in the spread of *B. anthracis*. While mechanical spread of *B. anthracis* organisms by stable flies has been demonstrated in guinea pigs (66), the importance of insects as vectors in epizootics has not been determined. One hypothesis suggests that insect bites might allow superficial organisms an effective access point for intradermal infection. Insects, particularly horseflies, were explicitly mentioned in 12 investigations for their possible role in transmission; however, no evidence exists that biting flies contribute to transmission of disease from animals to humans.

Past methods for decontaminating buildings relied upon formaldehyde gas, now known to be carcinogenic. The recent decontamination of *B. anthracis*–contaminated buildings was accomplished with chlorine dioxide gas, by using the methods developed for decontaminating textile mill buildings. Pre- and posttreatment environmental sampling strategies developed in several of the earlier field investigations, including the systematic use of surface swabs and spore strips, were also used in the response to recent events. In these events, the wide dispersion from envelopes of small airborne particles containing spores led to higher than expected levels of cross-contamination, making decontamination more difficult (65).

Several limitations should be considered in interpreting the results of this review. CDC conducts field investigations only when invited by a state health department or ministry of health. Anthrax cases that did not actively involve CDC staff, such as those investigated solely by state or local health departments, were excluded; therefore, this is not a complete report of U.S. anthrax case investigations. However, CDC staff have consulted at least by telephone on almost every case of human anthrax reported in the United States since the 1950s (A. Kaufmann, pers. comm.). A manuscript reviewing the characteristics of all anthrax cases reported in the United States since 1955 is in preparation (D. Ashford, pers. comm.).

Second, this review examines CDC's experience with field investigations involving anthrax; laboratory-based anthrax research was not included unless it was related to a field investigation. Third, final laboratory results were not available for some field investigations.

Conclusion

Much useful knowledge, ranging from the diagnosis of anthrax to the use of vaccine to protect populations, has been gained from these past investigations. However, many questions remain. Further research is needed to determine the lowest infectious dose, define what constitutes a true exposure for which antibiotic prophylaxis is warranted (especially in light of possible drug side effects), and determine whether spores delivered in an envelope create a residual risk after the primary contamination event. Other areas in which more research is needed include developing better rapid environmental testing methods (67), identifying optimal decontamination methods for a variety of contaminated settings, assessing *B. anthracis* spore background rates in selected settings, and determining the level of risk associated with a low degree of exposure to aerosols containing *B. anthracis*.

During the past 50 years, the scientific knowledge acquired in these field investigations has greatly improved the nation's ability to respond to anthrax outbreaks. New and unique challenges have been raised by the recent intentional release of *B. anthracis*. Further efforts to improve knowledge about anthrax, both in its natural setting and in the context of bioterrorism, are urgently needed.

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At the time this manuscript was prepared, Mr. Bales was an informatics research fellow in the Epidemiology Program Office at the Centers for Disease Control and Prevention, where he developed database systems for the Epidemic Intelligence Service. His research interests include developing conceptual models to represent and analyze knowledge derived from unstructured text and using geographic information systems to improve population health.

References

1. Centers for Disease Control and Prevention. Interim guidelines for investigation of and response to *Bacillus anthracis* exposures. MMWR Morb Mortal Wkly Rep 2001;50:987–90.
2. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. Am J Public Health 1962;52:632–45.
3. Kaufmann AF, Fox MD, Kolb RC. Anthrax in Louisiana, 1971: an evaluation of the Sterne strain anthrax vaccine. J Am Vet Med Assoc 1973;163:442–5.
4. Brachman PS, Fekety FR. Industrial anthrax. Ann N Y Acad Sci 1958;70:574–84.
5. Centers for Disease Control and Prevention. Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 2000;49(RR-15):1–20.
6. Tanner WB, Potter ME, Teclaw RF, Kaufmann AF, Bilderback WR, Dorris KD, et al. Public health aspects of anthrax vaccination of dairy cattle. J Am Vet Med Assoc 1978;173:1465–6.
7. Consumer Product Safety Commission. CPSC warns consumers of possible contamination of goatskin products. News release no.74-026, May 1974. Accessed July 8, 2002. Available from: URL: http://www.cpsc.gov/cpscpub/prerel/prhtml74/74026.html
8. Thacker SB, Dannenberg AL, Hamilton DH. Epidemic Intelligence Service of the Centers for Disease Control and Prevention: 50 years of training and service in applied epidemiology. Am J Epidemiol 2001;154:985–92.
9. Centers for Disease Control and Prevention. Public health emergency preparedness & response: anthrax. Accessed July 8, 2002. Available from: URL: http://www.bt.cdc.gov/Agent/Anthrax/Anthrax.asp
10. Centers for Disease Control and Prevention. Summary of notifiable diseases, United States, 1994. MMWR Morb Mortal Wkly Rep 1994;43:74,76,78.
11. Centers for Disease Control and Prevention. Summary of notifiable diseases, United States, 1999. MMWR Morb Mortal Wkly Rep 1999;48:84,86,88–9.
12. Suffin SC, Carnes WH, Kaufmann AF. Inhalation anthrax in a home craftsman. Hum Pathol 1978;9:594–7.
13. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001:7:1–22.
14. Centers for Disease Control and Prevention. Human anthrax associated with an epizootic among livestock—North Dakota, 2000. MMWR Morb Mortal Wkly Rep 2001;50:677–80.
15. National Institute for Occupational Safety and Health, Krake AM, Connon CL, Gomez TM. Health hazard evaluation report HETA 93-1121-2530, the State of North Dakota Department of Health and Consolidated Laboratories, Bismarck, North Dakota. Cincinnati (OH): The Institute; 1995. National Technical Information Service order number: PB96-197611.
16. Harrison LH, Ezzell JW, Abshire TG, Kidd S, Kaufmann AF. Evaluation of serologic tests for diagnosis of anthrax after an outbreak of cutaneous anthrax in Paraguay. J Infect Dis 1989;160:706–10.
17. Center for Disease Control. Animal anthrax—Texas. MMWR Morb Mortal Wkly Rep 1976;25:293.
18. Fox MD, Boyce JM, Kaufmann AF, Young JB, Whitford HW. An epizootiologic study of anthrax in Falls County, Texas. J Am Vet Med Assoc 1977;170:327–33.
19. Young JB. Epizootic of anthrax in Falls County, Texas. J Am Vet Med Assoc 1975;167:842–3.
20. Fox MD, Kaufmann AF, Zendel SA, Kolb RC, Songy CG Jr, Cangelosi DA, et al. Anthrax in Louisiana, 1971: epizootiologic study. J Am Vet Med Assoc 1973;163:446–51.
21. Center for Disease Control. Human anthrax—Louisiana. Morb Mortal Wkly Rep 1971;20:412.
22. Center for Disease Control. Cattle anthrax—Wyoming. Morb Mortal Wkly Rep 1970;19:389–90.
23. Communicable Disease Center. Animal anthrax and presumptive human anthrax—California. Morb Mortal Wkly Rep 1968;17:279.
24. Communicable Disease Center. Animal anthrax—North Dakota. Morb Mortal Wkly Rep 1965;14:270.
25. National Office of Vital Statistics. Anthrax in animals. Morb Mortal Wkly Rep 1957;6(34):2.
26. National Office of Vital Statistics. Anthrax. Wkly Morbidity Rep 1952;1(16):1.
27. Centers for Disease Control. Human cutaneous anthrax—North Carolina, 1987. MMWR Morb Mortal Wkly Rep 1988;37:413–4.

28. Young LS, Feeley JC, Brachman PS. Vaporized formaldehyde treatment of a textile mill contaminated with *Bacillus anthracis*. Arch Environ Health 1970;20:400–3.
29. Communicable Disease Center. Inhalation anthrax—Delaware County, Pennsylvania. Morb Mortal Wkly Rep 1961;10(28):2.
30. National Office of Vital Statistics. Provisional information on selected notifiable diseases in the United States and on deaths in selected cities for week ended March 5, 1960. Morb Mortal Wkly Rep 1960;9(9):1.
31. Brachman PS, Pagano JS, Albrink WS. Two cases of fatal inhalation anthrax, one associated with sarcoidosis. N Engl J Med 1961;265:203–8.
32. National Office of Vital Statistics. Anthrax. Morb Mortal Wkly Rep 1957;6(20):2.
33. Norman PS, Ray JG, Brachman PS, Plotkin SA, Pagano JS. Serologic testing for anthrax antibodies in workers in a goat hair processing mill. Am J Hyg 1960;72:32–7.
34. Brachman PS, Plotkin SA, Bumford FH, Atchison MM. An epidemic of inhalation anthrax: The first in the twentieth century. II. Epidemiology. Am J Hygiene 1960;72:6–23.
35. Dahlgren CM, Buchanan LM, Decker HM, Freed SW, Phillips CR, Brachman PS. *Bacillus anthracis* aerosols in goat hair processing mills. Am J Hygiene 1960;72:24–31.
36. Plotkin SA, Brachman PS, Utell M, Bumford FH, Atchison MM. An epidemic of inhalation anthrax, the first in the twentieth century. I. Clinical features. Am J Med 1960;29:992–1001.
37. National Office of Vital Statistics. Anthrax. Morb Mortal Wkly Rep 1956;5(13):1.
38. Centers for Disease Control and Prevention. Bioterrorism alleging use of anthrax and interim guidelines for management—United States, 1998. MMWR Morb Mortal Wkly Rep 1999;48:69–74.
39. Consumer Product Safety Commission. CPSC warns about possible anthrax contamination in imported yarn. News release # 76-011, February 1976. Accessed July 8, 2002. Available from: URL: http://www.cpsc.gov/cpscpub/prerel/prhtml76/76011.html
40. Center for Disease Control. Anthrax—California. Morb Mortal Wkly Rep 1976;25:33–4.
41. Occupational Safety and Health Review Commission and Administrative Law Judge Decisions. Docket number 76-596. Case citation 10 BNA OSHC 1203, 1981.
42. Communicable Disease Center. Cutaneous anthrax—New Jersey. Morb Mortal Wkly Rep 1965;14:308.
43. Communicable Disease Center. Case of anthrax—New Jersey. Morb Mortal Wkly Rep 1969;18:212.
44. Center for Disease Control. Animal anthrax associated with pack saddle pads—Washington. Morb Mortal Wkly Rep 1974;23:339–40.
45. Consumer Product Safety Commission. Consumers warned about possible saddle pad anthrax contamination. News release # 74-073, Nov 1974. Accessed July 8, 2002. Available from: URL: http://www.cpsc.gov/cpscpub/prerel/prhtml74/74073.html
46. Center for Disease Control. Anthrax contamination of Haitian goatskin products. MMWR Morb Mortal Wkly Rep 1977;26:31.
47. Center for Disease Control. Anthrax contamination of Haitian goatskin products. MMWR Morb Mortal Wkly Rep 1981;30:338.
48. Center for Disease Control. Cutaneous anthrax acquired from imported Haitian drums—Florida. MMWR Morb Mortal Wkly Rep 1974;23:142,147.
49. Center for Disease Control. Follow-up on cutaneous anthrax acquired from imported Haitian drums—Florida. MMWR Morb Mortal Wkly Rep 1974;23:149–50.
50. Center for Disease Control. Follow-up on cutaneous anthrax acquired from imported Haitian drums—Florida. MMWR Morb Mortal Wkly Rep 1974;23:224.
51. Communicable Disease Center. Anthrax—Ohio. MMWR Morb Mortal Wkly Rep 1964;13:118.
52. Roueche B. Annals of medicine: A man named Hoffman (original article published in 1965). The New Yorker. Posted Oct. 22, 2001 on the archive. Accessed July 8, 2002. Available from: URL: http://www.newyorker.com/FROM_THE_ARCHIVE/ARCHIVES/?011029fr_archive02
53. Communicable Disease Center. Cutaneous anthrax—South Carolina. Morb Mortal Wkly Rep 1965;14:270.
54. Centers for Disease Control and Prevention. Human ingestion of *Bacillus anthracis*-contaminated meat—Minnesota, August 2000. MMWR Morb Mortal Wkly Rep 2000;49:813–6.
55. Brachman PS, Kaufmann AF, Dalldorf FG. Industrial inhalation anthrax. Bacteriol Rev 1966;30:646–57.
56. Centers for Disease Control and Prevention. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:889–93.
57. Dixon TC, Meselson M, Guillemin J, Hanna PC. Medical progress: anthrax. N Engl J Med 1999;341:815–26.
58. Swartz MN. Recognition and management of anthrax—an update. N Engl J Med 2001;345:1621–6.
59. Center for Disease Control. Human orf mimicking cutaneous anthrax—California. MMWR Morb Mortal Wkly Rep 1973;22:108.
60. Carr EA, Rew RR. Recovery of *Bacillus anthracis* from the nose and throat of apparently healthy workers. J Infect Dis 1957;100:169–71.
61. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. Emerg Infect Dis 2002;8:1103-10.
62. Centers for Disease Control and Prevention. Additional options for preventive treatment for persons exposed to inhalation anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1142,1151.
63. Centers for Disease Control and Prevention. Update: interim recommendations for antimicrobial prophylaxis for children and breastfeeding mothers and treatment of children with anthrax. MMWR Morb Mortal Wkly Rep 2001;50:1014–6.
64. Brachman PS. Inhalation anthrax. Ann N Y Acad Sci 1980;353:83–93.
65. Centers for Disease Control and Prevention. Evaluation of *Bacillus anthracis* contamination inside the Brentwood Mail Processing and Distribution Center—District of Columbia, October 2001. MMWR Morb Mortal Wkly Rep 2001;50:1129–33.
66. Turell MJ, Knudson GB. Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). Infect Immun 1987;55:1859–61.
67. Centers for Disease Control and Prevention. Use of onsite technologies for rapidly assessing environmental *Bacillus anthracis* contamination on surfaces in buildings. MMWR Morb Mortal Wkly Rep 2001;50:1087.

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Night of Two Town Meetings

Matthew L. Cartter*

Driving south through Connecticut's Naugatuck River Valley brought back memories. My dad used to drive this road to and from work 30 years ago, when the river was actually more polluted but the circumstances were less complicated. On this night, a week after Thanksgiving, the fog seemed inseparable from the road. We were looking for Derby High School, site of our first town meeting. The high school was not far from the hospital where the day before Thanksgiving a 94-year-old woman from nearby Oxford had died of intentionally released inhalational anthrax.

As we pulled into the parking lot, more memories rushed into my mind—I came to this high school with my school's cross-country team back in 1970 to run a race. The 1970s seem so long ago—smallpox had been eradicated from the world, anthrax was a rare disease called woolsorters' disease, and the twin towers graced the New York City skyline. In those days, we would be visiting the 94-year-old Oxford woman to study the secrets of her longevity, to see how she had managed to beat life expectancy rates and survive to ripe old age. As our public health team made its way into the building, we were practically run over by a group of students using the hallway as an indoor track. I had to smile—I got my first shin splints running down school halls myself. Besides, it was heartening to see normal school activity in our changed world of post–September 11.

The local health director introduced me as the first of the evening's speakers to the town officials, legislators, first responders, and a score of townspeople who had braved the foggy evening to attend the meeting. In spite of the bright lights, a different kind of fog hung heavily inside the building. As I approached the podium, I felt the expectations of the people in the audience. They needed specific, practical, information about their predicament as a small community in the middle of a disease outbreak caused by bioterrorism. I had been to many town meetings before, but this meeting was different. In my public health career until now, disease outbreaks had been natural events. Investigators and the community had a defined enemy, a disease. Now, the disease was only part of the problem. Someone had intentionally created this outbreak, and while we knew how to go after viruses and bacteria, we knew little about human perpetrators of disease.

"I am a public health physician involved in the anthrax investigation," I offered, "here to discuss the 22 cases of anthrax reported in the United States over the past 2 months. I will also talk about how to handle your mail." My presentation covered the history of the anthrax outbreaks in the District of Columbia, Florida, New Jersey, and New York; the findings of the outbreak investigations; and the findings of the criminal investigations reported in the press. In addition to the usual slides shown by epidemiologists during traditional outbreaks, I showed slides of the letters sent through the postal service to newsman Tom Brokaw and to Senator Daschle. The Brokaw letter had no return address, I elaborated, while the return address on the Daschle letter was a fictitious New Jersey elementary school. I could tell that many in the audience had not looked closely at these handwritten letters before (handwriting is personal and adds to the abomination of these letters).

When my talk came to a close, I explained that those of us from the state health department needed to leave for a town meeting in Oxford. I thanked the audience and the other speakers for their understanding. I heard several people say thanks as I walked up the aisle. I have never had to leave a town meeting early and hoped that things would go well for those talking and answering questions after me.

The fog thickened as we headed out of the valley to a middle school in Oxford. Here, the parking lot was filled with cars and media trucks with satellite dishes, signs of the media frenzy that permeated the anthrax investigation. The meeting was already well under way. I looked through a small window in the door of the gymnasium and took a deep breath before entering—town meetings are unscripted events that can be very unpredictable, especially when the media are present. The public and the media were seated in chairs and bleachers on one side of the room. The speakers, finished with their presentations, were seated at tables on the opposite side. The local health director was standing at the podium responding to questions. As we walked through the door, he seemed relieved to introduce us as "the folks from the state health department." I sensed that the tension I felt was not all mine.

On my way to the podium, I passed the speakers' table and nodded to the physician who had treated the elderly anthrax patient. I greeted the First Selectman, who was completing her second week in office. Earlier in the day, she had mentioned to me that some town residents still hoped that this was a case of "natural" anthrax, not connected to recent events. I knew that some in the audience would not be comforted by what I needed to say.

"I am a physician from the state health department," I began, "and have been part of many public health investigations: Lyme disease, West Nile virus infection, and now anthrax. I grew up not far from here, in a small town much like Oxford, and my job today is to answer your questions."

One man wanted to know if we had found anthrax in any of the soil samples we had collected; another how long anthrax

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from contaminated letters remained viable. A woman asked if it was okay for her children to touch the mail. The concerns and questions were many and far-reaching, but I had settled in and did not feel a need to hurry. The press seemed anxious to meet their deadlines, but this meeting was not for them. Earlier in the afternoon, a report had been released on the status of the anthrax investigation. I gave a brief analysis of the report. The source of exposure to *Bacillus anthracis* for the elderly Connecticut resident remains unknown, the report said. The genetic characteristics of *B. anthracis* isolated from the patient were similar to those found in other bioterrorism-related cases; however, the epidemiologic characteristics and the potential sources of exposure were different (1).

"Although we will probably never know exactly how your elderly neighbor became infected," I explained, "she was probably exposed to mail contaminated with anthrax spores. The mail threat, at least this episode, will pass with time, but those of you who live in Oxford, where she lived, may never feel the same about opening the mail."

As the questions subsided and the meeting came to a close, I made my way through the circle of officials and the dwindling crowd to the door, where the reporters awaited with questions about the newly released report. Finally outside the building, I felt the cool evening air with relief. Even the fog seemed less ominous. "How did it go?" asked one of my colleagues from the state health department as we walked away from the school. "These folks have been through a lot," I said, "and I feel privileged to be here."

I left the world of clinical medicine 18 years ago and went to work at the state health department in Connecticut, at first, as part of Centers for Disease Control and Prevention's Epidemic Intelligence Service. A few years later, a friend gave me an article written by a physician about what it can be like to work in public health (2). I have given a copy of this article (A Piece of My Mind. Have You Ever Practiced Medicine?) to all medical epidemiologists I have supervised.

On the way home from Derby and Oxford, I felt proud to be part of the public health response to bioterrorism. I thought about how physicians in public health still struggle, on occasion, with the question, "Have you ever practiced medicine?" On the night of two town meetings, I knew that the answer was "yes" to the question, "Have you ever practiced public health?"

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References

1. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related inhalational anthrax—Connecticut, 2001. MMWR 2001;50:1049–51.
2. Dandoy S. A piece of my mind. Have you ever practiced medicine? JAMA 1988;260;2113.

EMERGING INFECTIOUS DISEASES www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and *West Nile virus* infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

Evaluation and Validation of a Real-Time Polymerase Chain Reaction Assay for Rapid Identification of *Bacillus anthracis*

To the Editor: During the 2001 anthrax outbreak, we evaluated and validated a highly sensitive and specific three-target (two plasmid and one chromosomally located target) 5′ nuclease assay (real-time polymerase chain reaction [PCR]) for detection and identification of *Bacillus anthracis*. This PCR assay was successfully used to rapidly test hundreds of suspect isolates as well as screen environmental samples for the presence of *B. anthracis* throughout the 2001 anthrax outbreak. For the first time in an outbreak setting, a PCR assay was used to detect *B. anthracis* directly from clinical specimens, consequently becoming a part of the laboratory confirmation of anthrax. In this letter, we describe the evaluation of this assay on a diverse panel of bacterial isolates including isolates obtained throughout the outbreak. A supplement, which includes data on the use of this assay on environmental and clinical specimens, is online (available from: URL: http://www.cdc.gov/ncid/EID/vol8no10/02-0393_sup.htm).

Identification of *B. anthracis* has traditionally been determined by using phenotypic differences between *B. anthracis* and the rest of the *B. cereus* group (i.e., lack of motility and hemolysis, susceptibility to penicillin, typical colony morphology, and susceptibility to lysis by gamma phage); however, these methods are slow and require at least 24 h for completion. The recent bioterrorism-associated outbreak and the ongoing threat emphasize the importance of rapid microbiologic diagnosis for the timely and adequate implementation of control and preventative measures.

For *B. anthracis*, the main targets for development of such assays, primarily PCR-based, have been and continue to be genes encoding its virulence factors: a tripartite exotoxin and an antiphagocytic capsule (1–4). The toxin genes (*pag*A, *lef*, and *cya*) are encoded on the 182-kb virulence plasmid, pXO1, while the genes required for capsule biosynthesis (*capB*, *capC*, and *capA*) are encoded on the 96-kb virulence plasmid, pXO2 (5–7). These plasmid-located virulence genes seem to be restricted to *B. anthracis*, giving the plasmid-based assays a high degree of specificity (8). However, strains of *B. anthracis* that lack these plasmids have been reported (4,9). Consequently, having an assay focus on a specific chromosomal target for detection of avirulent and plasmid-cured *B. anthracis*, as well as those that potentially could have been genetically engineered, is essential. Chromosomal markers, such as *vrrA* and Ba813, have been used to characterize *B. anthracis* (9–12) and to detect it in tissues of victims of the anthrax outbreak that occurred in 1979 in Sverdlovsk, former Soviet Union (12), but these markers are not restricted to *B. anthracis*. Recently, Qi et al. developed a fluorescence resonance energy transfer PCR assay that targets the *B. anthracis* chromosomally located *rpoB* gene. This assay appears to be the most specific described to date with only 1 of 175 non-*B. anthracis* bacilli reported as positive (13).

Over the past several years, activities in the area of bioterrorism preparedness in the United States have resulted in the establishment of an international Laboratory Response Network (LRN), which was instrumental in the identification of the agent used in the 2001 outbreak (14). One of the major initiatives of LRN has been development and validation of rapid and specific assays for identification of *B. anthracis* and other agents likely to be used in a bioterrorism event.

Primer and probe set BA1 targets a region of pX02, BA2 targets pXO1, and BA3 targets a region of the *B. anthracis* chromosome. Probes were labeled with 6-carboxy-fluorescein phosphoramidite and 5-carboxy-tetramethyl-rhodamine.

LRN PCR assays using the BA1, BA2, and BA3 primer and probe sets were performed with the LightCycler (Roche Diagnostics GmbH, Mannheim, Germany), Smart Cycler (Cepheid, Sunnyvale, CA), or ABI Prism 7700 (Applied Biosystems, Foster City, CA) instruments. The LightCycler Faststart DNA master hybridization probes kit (Roche Diagnostics GmbH) reagents were used on all real-time platforms. Reactions comprised 1X reaction mix, 5 mM $MgCl_2$, 500 nM each primer, and 100 nM probe in a reaction volume of 20 µL (LightCycler) or 25 µL (Smart Cycler, ABI Prism 7700). Thermal cycler conditions consisted of an initial 10-min hold at 95°C followed by 40–45 cycles of 10 s (LightCycler) or 15 s (Smart Cycler, ABI Prism 7700) at 95°C and 30 s (LightCycler, Smart Cycler) or 60 s (ABI Prism 7700) at 60°C. Real-time data were collected during the 60°C extension step of each cycle. Amplification of the human β-actin gene was used as a real-time PCR control when used in clinical samples to ensure negative results were not from inhibition of the PCR reaction. This real-time PCR assay was considered positive when all three targets were positive (Figure).

A total of 542 isolates were tested. Eighty-one *B. anthracis* isolates were tested to evaluate sensitivity of the real-time PCR approach (Table). Seventy-five were selected to provide a test population representing diverse sources, genotypes, geographic origins, and dates of isolation. The isolates included those collected from animals, humans, and other sources (i.e., industrial sites associated with anthrax outbreaks); the isolates span at least 58 years (1939–1997). Fifty-three of the isolates were previously characterized by multiple-locus variable-number tandem repeat analysis (MLVA) (15) and were included to ensure a representative range of the 89 described MLVA genotypes to date.

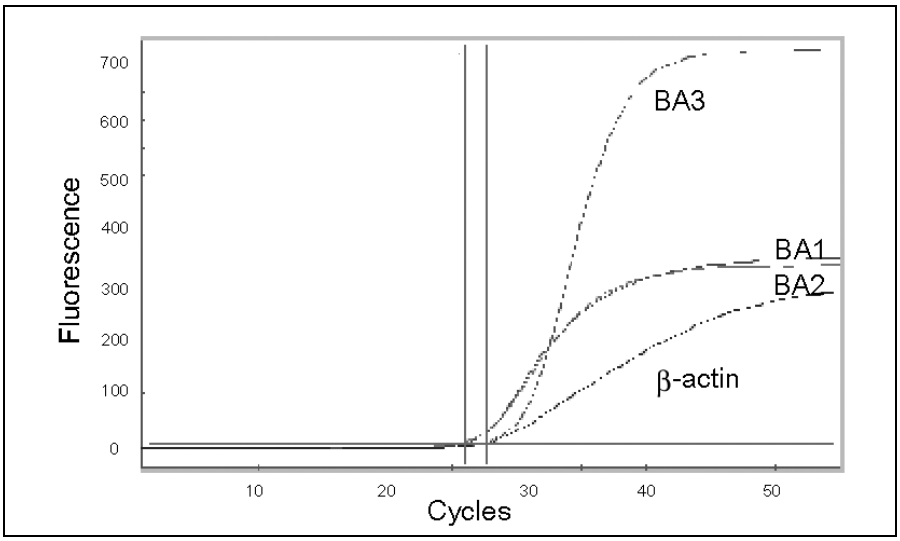

Figure. Real-time polymerase chain reaction graph of three *Bacillus anthracis* markers and β-actin control detected in a pleural fluid specimen from a patient with inhalational anthrax. The horizontal line indicates a threshold value; the vertical lines indicate cross-threshold values for each marker. BA1, primer and probe set targeting a region of pXO2; BA2, primer and probe set targeting a region of pXO1; BA3, primer/probe set targeting a region of *B. anthracis* chromosome.

Six *B. anthracis* type and standard strains included: five pXO1 cured strains (including the Pasteur strain) and one pXO2 cured strain (the veterinary vaccine strain Sterne). The *B. anthracis* New Hampshire strain (16) was used as a positive control for all real-time PCR assays. This isolate was originally cultured from a patient with inhalational anthrax in New Hampshire in 1957. This real-time PCR is designed to identify fully virulent (wild-type) *B. anthracis,* which will give positive results in all three markers. However, naturally occurring isolates have been found lacking either virulence plasmid, and a number of laboratory strains have been plasmid cured, as well. PCR results for these strains will reflect the lack of one or both of their plasmids.

A total of 317 *B. anthracis* isolates obtained during the bioterrorism-associated anthrax outbreak from October to December 2001 were also analyzed by PCR. These included 27 isolates from clinical specimens, 4 from powders and 286 isolates from environmental samples. MLVA was performed on 135 of these isolates; all were indistinguishable (17).

For evaluation of the assays' specificity we tested 56 archived members of the *Bacillus* genus: *B. subtilis* (9 strains, 5 clinical, 4 unknown), *B. cereus* (23 strains, 9 clinical, 14 environmental), *B. thuringiensis* (12 strains, 6 clinical, 3 insects, 3 unknown), *B. mycoides* (1 strain, unknown), *B. megaterium* (10 strains, 7 clinical, 3 unknown), and the environmental *Bacillus* spp. isolate, Ba813_11, which resulted in a previously reported false-positive result in the *B. anthracis*-specific PCR assay targeting *rpoB* (13). In addition, 88 isolates from environmental and clinical specimens, which were confirmed not to be *B. anthracis* by standard microbiologic methods were tested. These isolates were selected because of their lack of hemolysis and because they had a colony morphology similar to *B. anthracis* on blood agar plates.

Before testing, all strains were stored at –70°C in brain heart infusion broth (BHIB, Centers for Disease Control and Prevention [CDC], Atlanta, GA) or water containing 20% glycerol. Identification of all strains was confirmed by using standard microbiologic procedures and the LRN testing algorithm (14,18). Colony-lysis DNA preparations were used for all *Bacillus* spp. strains. Isolates were streaked onto trypticase soy agar containing 5% sheep blood (Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated overnight at 37°C. A single colony was transferred and dispersed into 0.22 μM centrifugal filter units (Millipore, Bedford, MA) containing 200 μL 10 mM Tris-HCl (pH 8.0). The suspension was heated at 95°C for 20 min and then cooled to room temperature. The filter units were then centrifuged at 6,000 x *g* in a microfuge for 2 min and the filter discarded. The resulting lysate was stored at –20°C until use.

The lower limit of detection of each assay was tested by using five *B. anthracis* strains: Ames (2000 031656), Pakistan-sheep (20000316 48), French-bovine (2000031651), Sterne (2000031075), and Pasteur (20 00031759). DNA was extracted from vegetative cells by first pre-treating cell pellets with lysozyme and lysostaphin and then using the MasterPure DNA Purification kit (Epicentre, Madison, WI), following the manufacturer's protocol for cell samples. *B. anthracis* spores were quantitated microscopically and tested directly in the real-time PCR assay without DNA extraction. Vegetative-cell DNA was tested at concentrations ranging from 10 ng to 400 fg DNA per reaction. Spores were tested at concentrations ranging from 100,000 spores to 1 spore per reaction. All reactions were performed in duplicate on the LightCycler, Smart Cycler, and ABI Prism 7700 instruments.

All 75 wild-type (fully virulent) *B. anthracis* isolates tested were positive for all three targets resulting in 100% sensitivity (95% confidence interval [CI] 95% to 100%). Strains cured of pXO1 or pXO2 produced negative results for the loci specific to these plasmids (Table). In addition, all 317 *B. anthracis* isolates from the 2001 outbreak were also positive for all three PCR targets (Table).

None of the 56 archived non–*B. anthracis* isolates, representing five other *Bacillus* species was positive for any of the three LRN PCR targets,

Table. Origin, designations, and results of real-time polymerase chain reaction assay for *Bacillus anthracis* strains

B. anthracis	No. analyzed	Temporal range and geographic origin	MLVA genotypes represented[a]	No. positive/total		
				Ba1[b]	Ba2[b]	Ba3[b]
Human isolates	30	1943–1996 Africa, Asia, Australia, Europe, North America	3, 4, 22, 23, 28, 32, 34, 35, 36, 37, 41, 43, 44, 45, 50, 66, 68	30/30	30/30	30/30
Animal isolates	29	1939–1997 Africa, Asia, Australia, Europe, North America, South America	3, 10, 20, 26, 29, 30, 35, 38, 40, 45, 48, 49, 51, 55, 57, 78, 80, 81, 84, 85, 87, 89	29/29	29/29	29/29
Other isolates	16	1950–1993 Africa, Asia, Europe, N. America	13, 14, 21, 24, 47, 62, 69, 73, 77, 79, 82	16/16	16/16	16/16
Outbreak isolates	317	2001 U.S. outbreak	62	317/317	317/317	317/317
pXO1 cured	5	1956–1974 North America		5/5	0/5	5/5
pXO2 cured	1	Africa		0/1	1/1	1/1

aMLVA, multiple-locus variable-number tandem repeat analysis as described by Keim et al. (15).
bBa1, Ba2, and Ba3 primer/probe sets as described in Materials and Methods.

including the *Bacillus* spp. isolate, Ba813_11, resulting in 100% specificity (95% CI 94% to 100%). Results were also negative for 88 clinical and environmental isolates, which were determined by standard microbiologic methods not to be *B. anthracis* (specificity 100%, 95% CI 96% to 100%).

The limit of detection on the LightCycler, Smart Cycler, and ABI Prism 7700 instruments, as determined by using DNA extracted from vegetative cells of the Sterne and Pasteur reference strains, was 1 pg DNA (approximately 167 cells based on a 5.5 Mbp genome size). Five to 10 spores could be detected on the ABI Prism 7700 instrument for the Ames (2000031656), Pakistan-sheep (20000 31648), French-bovine (2000031651), and Sterne (2000031075) strains of *B. anthracis*.

The recent bioterrorism-associated anthrax outbreak demonstrated the need for sensitive, specific, and rapid methods for diagnosis and confirmation of anthrax, both for identification of suspect *B. anthracis* isolates and direct detection of *B. anthracis* DNA in clinical specimens. When tested on >500 strains, representing *B. anthracis* and five other *Bacillus* species, the LRN PCR exhibited 100% sensitivity and specificity.

To date, designing PCR assays for identification of *B. anthracis* has primarily focused on genes located on the plasmids (1–4). Patra et al. used a PCR that targeted two chromosomal loci, *vrrA* and Ba813, and found numerous environmental *Bacillus* isolates other than *B. anthracis* that were positive for both Ba813 and *vrrA* (11). While assays focusing on plasmid targets allow for a high level of specificity, a specific chromosomal target for detection of avirulent and plasmid-cured *B. anthracis* strains is needed. Thus, the LRN PCR includes a chromosomal target in addition to targets on each of the two virulence plasmids, pXO1 and pXO2.

Closely related *B. cereus* and *B. thuringiensis*, notorious for generating false-positive results using assays designed to be specific for *B. anthracis* (11,13), were consistently negative in this real-time PCR assay. *B. anthracis, B. cereus*, and *B. thuringiensis* are so closely related that their distinction as separate species is frequently questioned based on DNA-DNA hybridization studies, multiple-locus enzyme electrophoresis, and 16S rRNA sequence similarity (19–21). We have selected non–*B. anthracis* isolates that were primarily of clinical as opposed to environmental origin. *B. cereus* and *B. thuringiensis* clinical isolates are even more closely related to *B. anthracis* than their environmental counterparts (19,22), and they are more likely to cause false-positive results. We also tested the *Bacillus* spp. isolate that caused the one false-positive result in the Qi et al. report (13). Despite all of these challenges, all three targets of this real-time PCR assay have demonstrated 100% specificity and sensitivity in identification of *B. anthracis* when tested against our panel of *Bacillus* spp. strains and in identification of 317 outbreak-associated *B. anthracis* isolates. This LRN PCR is currently the only real-time PCR assay that detects both plasmid and chromosomal targets with 100% specificity and sensitivity. In addition, real-time PCR assays using fluorescent probes provide great sensitivity; this assay was able to detect 1 pg of purified DNA from vegetative cells (equivalent to 167 cells) or directly detect 5–10 spores.

The high level of sensitivity and specificity of the LRN PCR assay can be attributed to several factors. An extensive panel of DNA samples (non-*Bacillus* gram-positive bacterial species, gram-negative bacterial species, and human, vertebrate, and invertebrate DNA) were tested (data not shown). Having more than a single target decreases the rate of both false-negative and false-positive results, as they are not dependent on a single locus. The use of multiple targets also decreases the risk of false-positive results from contamination because each target is amplified as a separate PCR reaction. Finally, 5′ nuclease

assays makes use of a fluorescent oligonucleotide probe, in addition to the forward and reverse primers, that allows for a lower limit of detection compared to conventional PCR, eliminates the need for post-PCR processing, and increases specificity (23,24).

The LRN PCR was shown to be important for use on environmental and clinical specimens during the 2001 bioterrorism-associated anthrax outbreak. A supplement covering the use of this assay on these specimens can be seen online (available from: URL: http://www.cdc.gov/ncid/EID/vol8no10/02-0393_sup.htm). The LRN PCR assay is widely available at over 200 laboratories in several countries and all 50 states of the United States through the Laboratory Response Network. The system is designed to be accessed through the State Department of Health.

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References

1. Makino SI, Cheun HI, Watarai M, Uchida I, Takeshi K. Detection of anthrax spores from the air by real-time PCR. Lett Appl Microbiol 2001;33:237–40.
2. Makino SI, Iinuma-Okada Y, Maruyama T, Ezaki T, Sasakawa C, Yoshikawa M. Direct detection of *Bacillus anthracis* DNA in animals by polymerase chain reaction. J Clin Microbiol 1993;31:547–51.
3. Lee MA, Brightwell G, Leslie D, Bird H, Hamilton A. Fluorescent detection techniques for real-time multiplex strand specific detection of *Bacillus anthracis* using rapid PCR. J Appl Microbiol 1999;87:218–23.
4. Turnbull PC, Hutson RA, Ward MJ, Jones MN, Quinn CP, Finnie NJ, et al. *Bacillus anthracis* but not always anthrax. J Appl Bacteriol 1992;72:21–8.
5. Okinaka RT, Cloud K, Hampton O, Hoffmaster AR, Hill KK, Keim P, et al. Sequence and organization of pXO1, the large *Bacillus anthracis* plasmid harboring the anthrax toxin genes. J Bacteriol 1999;181:6509–15.
6. Okinaka RT, Cloud K, Hampton O, Hoffmaster AR, Hill KK, Keim P, et al. Sequence, assembly and analysis of pXO1 and pXO2. J Appl Microbiol 1999;87:261–2.
7. Green BD, Battisti L, Koehler TM, Thorne CB, Ivins BE. Demonstration of a capsule plasmid in *Bacillus anthracis*. Infect Immun 1985;49:291–7.
8. Pannucci J, Okinaka RT, Sabin R, Kuske CR. *Bacillus anthracis* pXO1 plasmid sequence conservation among closely related bacterial species. J Bacteriol 2002;184:134–41.
9. Ramisse V, Patra G, Garrigue H, Guesdon GL, Mock M. Identification and characterization of *Bacillus anthracis* by multiplex PCR analysis of sequences on plasmids pXO1 and pXO2 and chromosomal DNA. FEMS Microbiol Lett 1996;145:9–16.
10. Ramisse V, Patra G, Vaissaire J, Mock M. The Ba813 chromosomal DNA sequence effectively traces the whole *Bacillus anthracis* community. J Appl Microbiol 1999;87:224–8.
11. Patra G, Vaissaire J, Weber-Levy M, Le Doujet C, Mock M. Molecular characterization of *Bacillus* strains involved in outbreaks of anthrax in France in 1997. J Clin Microbiol 1998;36:3412–4.
12. Jackson PJ, Hugh-Jones ME, Adair DM, Green G, Hill KK, Kuske CR, 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;95:1224–9.
13. Qi Y, Patra G, Liang X, Williams LE, Rose S, Redkar RJ, et al. Utilization of the *rpoB* gene as a specific chromosomal marker for real-time PCR detection of *Bacillus anthracis*. Appl Environ Microbiol 2001;67:3720–7.
14. Khan AS, Morse S, Lillibridge SR. Public-health preparedness for biological terrorism in the USA. Lancet 2000;356:1179–82.
15. Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. J Bacteriol 2000;182:2928–36.
16. Plotkin SA, Brachman PS, Utell M, Bumford FH, Atchison MM. An epidemic of inhalation anthrax, the first in the twentieth century. Am J Med 1960;29:992–1001.
17. Hoffmaster AR, Fitzgerald CC, Ribot E, Mayer LW, Popovic T. Molecular subtyping of *Bacillus anthracis* and the 2001 bioterrorism-associated anthrax outbreak, United States. Emerg Infect Dis 2002;8;1111-6.
18. Logan NA, Turnbull PC. *Bacillus* and recently derived genera. In: Murray PR, editor. Manual of clinical microbiology. Washington: ASM Press; 2001. p. 357–69.
19. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, et al. *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*—one species on the basis of genetic evidence. Appl Environ Microbiol 2000;66:2627–30.
20. Ash C, Farrow JA, Dorsch M, Stackebrandt E, Collins MD. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse tran-

scriptase sequencing of 16S rRNA. Int J Syst Bacteriol 1991;41:343–6.
21. Kaneko T, Nozaki R, Aizawa K. Deoxyribonucleic acid relatedness between *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis*. Microbiol Immunol 1978;22:639–41.
22. Ticknor LO, Kolsto AB, Hill KK, Keim P, Laker MT, Tonks M, et al. Fluorescent amplified fragment length polymorphism analysis of Norwegian *Bacillus cereus* and *Bacillus thuringiensis* soil isolates. Appl Environ Microbiol 2001;67:4863–73.
23. Cockerill FR, Smith TF. Rapid-cycle real-time PCR: a revolution for clinical microbiology. ASM News 2002;68:77–83.
24. Livak KJ, Flood SJ, Marmaro J, Giusti W, Deetz K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. PCR Methods Appl 1995;4:357–362.

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Industry-Related Outbreak of Human Anthrax, Massachusetts, 1868

To the Editor: In Bioterrorism-Related Inhalational Anthrax: The First 10 Cases Reported in the United States, Jernigan et al. noted that in the mid-1800s inhalational anthrax related to the textile industry became known as woolsorters' disease (in England) and ragpickers' disease (in Germany and Austria) because of the frequency of infection in mill workers exposed to imported animal fibers contaminated with *Bacillus anthracis* spores (1). During the 1800s, as in Europe, industry-related human cases of anthrax also occurred in the United States.

In 1868, Silas Stone, a physician, reported that "an unusual number of cases of a rather rare affection have come under my observation within the past 14 months" (2). Stone described eight patients with "malignant pustules" who worked in or were associated with an animal hair factory in Massachusetts. The patients' cutaneous lesions were described as dark red, dark purple, purplish-black, and black; six of the patients had "slough" lesions. Stone treated his patients with tincture of iodine, iron, and quinine. Since antibiotics were not available, six of the eight patients had severe clinical disease, and two died. Stone's patients demonstrated the full spectrum of anthrax, including gastrointestinal, mediastinal, and meningeal involvement. Four patients had gastrointestinal symptoms, including epigastric distress and pain, nausea, and vomiting. Three patients had mediastinal involvement, manifested by chest distress and pain, dyspnea, and tachypnea. In the two fatal cases, meningitis appeared to have been the immediate cause of death; both of these patients were described as delirious.

Among Stone's eight patients, most remarkable was case 5, which was strikingly similar to case 8 of Jernigan et al.; the signs and symptoms of both patients included chills, headache, fatigue, vomiting, chest pain, tachypnea, tachycardia, and cutaneous lesions. Stone's description of the 7-day clinical course of patient 5, a laborer at the hair factory, is as follows: "Called November 17. Had been sick since the Thursday previous (November 14). Was taken with chills, pain in head and back, and suffered loss of strength. When first seen, was in bed . . . had not slept well the previous night. Pain and distress in epigastrium and back. Pulse 120 . . . breathing hurried. Discovered a dark purple spot surrounded by yellow vesicles . . . pressure on slough produced no pain. November 18: Slough doubled in size. November 19: Vomited . . . severe chill. November 20: Sleep restless . . . slough one inch by half an inch, much raised above surrounding skin, with a red areola about an inch in width. November 21: a.m.: Delirious part of night . . . slept but little . . . pain in chest. 3 p.m.: Distress at epigastrium great . . . delirium more violent. 8 p.m.: Distress and delirium greater . . . pulse failing . . . sinking rapidly . . . died soon after visit."

Stone perceptively noted that each of his patients was directly or indirectly exposed to hair or dirt from the animal hair factory, and that in the surrounding population not so exposed, no cases were seen. Stone realized that he was dealing with an industry-related disease and hypothesized that the cause was "a specific poison, and not simply putrescent animal matter." Nine years after Stone's 1868 report, Robert Koch in Germany reported isolation and cultivation of *B. anthracis*, the formation of its spores, the production of anthrax disease with pure cultures, and the recovery of *B. anthracis* from experimental infection (3).

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References

1. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: The first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44.
2. Stone SE. Cases of malignant pustule. Boston Medical and Surgical Journal 1868;I:19–21.
3. Jay V. The legacy of Robert Koch. Arch Pathol Lab Med 2001;125:1148–9

Meeting Summary

Bioterrorism-Related Public Health *Bacillus anthracis* Research Priorities

During the 2001 anthrax bioterrorism investigation, several areas were identified in which additional research may improve public health response. The disciplines and specific expertise required to approach many of these areas are varied and reside in multiple entities in the federal government and elsewhere. To identify, prioritize, and coordinate short-term *Bacillus anthracis* bioterrorism research for public health response, the Centers for Disease Control and Prevention (CDC) convened a meeting in Atlanta on December 10–11, 2001, to obtain input on research priorities and improve coordination with federal partners and other stakeholders. One hundred thirty-two representatives from the Department of Health and Human Services (CDC, Food and Drug Administration, and National Institutes of Health), Environmental Protection Agency, Department of Defense, Department of Energy, Department of Justice, U.S. Postal Service, state health departments, universities, and other organizations participated in the meeting.

The meeting format consisted of two plenary sessions. In the first plenary session experts provided summaries of key topic areas. Background talks were given on evaluation of *B. anthracis*–containing powders or substances; epidemiologic investigation; environmental assessment; surveillance; diagnosis; treatment; postexposure prophylaxis; and remediation.

After the first plenary session, participants were divided into eight preassigned working groups, covering the following topics: 1) evaluation of *B. anthracis*–containing powders or substances; 2) epidemiologic investigation; 3) environmental assessment; 4) surveillance; 5) diagnosis; 6) treatment; 7) postexposure prophylaxis; and 8) remediation. During the second plenary session, each group presented interim results to the larger group of participants. In two working group sessions, the topic-specific groups identified their three top research priorities and prepared a brief written report describing proposed activities.

For evaluation of *B. anthracis*–containing powders or substances, the top three priorities were 1) rapid analysis of anthrax-containing powder: particle size distribution and matrix characteristics; 2) measurement of particle reaerosolization of different anthrax powder preparations, and 3) development of an in vitro model for the study of cutaneous anthrax by human cell culture. For epidemiologic investigation, priorities were 1) analysis of individual host risk factors for anthrax infection; 2) exposure reconstruction and risk characterization; and 3) review of unexamined or previously unpublished (potentially classified) animal data related to dose response. For environmental assessment, the top priorities were 1) validation and standardization of sampling and sample analysis techniques; 2) evaluation of risk of disease in contaminated environments; and 3) determination of risk of reaerosolization. For surveillance, priorities were 1) expanded veterinary surveillance and integration with human health information; 2) use of alternative sources of data in the surveillance for bioterrorism-related events; and 3) design and validation of surveillance systems to detect complex contamination or release scenarios. For diagnosis, priorities were 1) identification of the earliest detectable event in the continuum from exposure to anthrax to disease (using animal models); 2) evaluation of antigen-detection assays; and 3) development of a library of *B. anthracis* subtypes. For treatment, priorities were 1) investigation of the role of immune and antitoxin therapies; 2) expanded investigation of antibiotic therapies in animal models; and 3) development of other animal models. For postexposure prophylaxis, priorities were 1) evaluation of adherence, barriers to adherence, and adverse events associated with long-term use of antimicrobial agents; 2) pediatric anthrax vaccine safety and immunogenicity studies; and 3) animal challenge studies to optimize postexposure prophylaxis in humans. For remediation, the top three priorities were 1) evaluation of remediation agents; 2) development of risk-based decision templates for sampling and remediation; and 3) reaerosolization studies and agent- and space-specific scenarios.

This meeting defined a framework and set specific priorities for additional research needed to improve public health response to *B. anthracis*–related bioterrorism. Explanations of why the research was considered important, what the research would be, how it would be carried out, who could do it, and when it could begin are available online from URL: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/files/MeetingReport _BTPriorities_Dec1011.pdf

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About the Cover

Norman Rockwell (1894–1978). Postman Reading Mail (Saturday Evening Post cover, 18 February 1922). Courtesy of the Curtis Publishing Company, Indianapolis, Indiana, USA.

Norman Rockwell, North America's most beloved and certainly best known illustrator, favored scenes of everyday life and reveled in his ability to tell stories. His Dickensian view of life drove him to paint the world as he would like it to be—no drunken fathers or self-centered mothers, only kindly doctors, duty-bound soldiers, and regular folks at their daily occupations. In his pictures, the sadness was wistful and the problems humorous. Rockwell, who presented himself as an illustrator rather than a fine arts painter, was also an interpreter of the classics and a recorder of history and the contemporary scene, from the Nuclear Test Ban Treaty to the Civil Rights Movement (1).

The realism in Rockwell's illustrations was not photographic. Along with the artful detail, his cast of characters (teachers, students, models, homemakers) was loaded with nuances bestowed by the illustrator's genius. The characters sparkled, glowed, and communicated directly with the audience. And the message they sent was exactly the one Rockwell intended the audience to receive. Very much in touch with his surroundings, the artist lived many of the situations that eventually became the subjects of his pictures. He painted what he knew to be the life of a child, a student, a soldier, or a workingman, whether in the Mamaroneck, New York, of his youth, or anywhere else he lived after that. As a result, his characters were universal and accessible to the average viewer.

Rockwell's brief experience with the U.S. Postal Service, which may have colored his many depictions of postal workers of all ages, was in the eighth grade. To raise money for art school tuition, he bought the mail route to exclusive Orienta Point from another boy for $25. The wealthy residents of the Point each paid the mail carrier 25 cents to deliver the mail because the regular carrier did not deliver that far from town. Every morning at 5:30, rain or shine, Norman bicycled to the post office, loaded the mail into a leather shoulder bag, and rode 2.5 miles to the end of the Point, delivering the mail to homes on the way.

When "Postman Reading Mail" first hit the stands on the cover of the Saturday Evening Post, thousands of letters from postal workers protested the nosy behavior ascribed to one of their own. Rockwell fielded the protests graciously, explaining that the post office was a small operation, in a tiny town, with a few boxes of mail to sort, and the postal clerk had succumbed to boredom and human curiosity: "…if you are interested in the characters that you draw, and understand them and love them, why the person who sees your picture is bound to feel the same way." (Curtis Publishing Co. comm., 2002).

If premier illustrator Norman Rockwell were alive today, he would be painting a different mail scene from the one featured on the cover of the Saturday Evening Post in 1922 and now on the cover of Emerging Infectious Diseases. Under the current circumstances of the world, in which the routine and harmless activity of sorting and delivering the mail was deliberately contaminated with a dreaded disease, this fine recorder of history would probably forego the humor in the scene.

Polyxeni Potter

1.Rockwell N. My Adventures as an Illustrator. Harry Abrams, Inc. New York. 1988.

EMERGING INFECTIOUS DISEASES
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Upcoming Issue on Tuberculosis

For a complete list of articles included in the November issue, and for articles published online ahead of print publication, see http://www.cdc.gov/ncidod/eid/upcoming.htm

Look in the November issue on Tuberculosis for the following topics:

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Cross-Jurisdictional Transmission of *Mycobacterium tuberculosis* in Maryland and Washington, D.C., 1996–2000, Linked to the Homeless

Molecular Epidemiology of Multidrug-Resistant Tuberculosis, New York City, 1995–1997

Transmission of an Endemic Strain of *Mycobacterium tuberculosis* in a Rural Community, 1945–2000

Human Exposure After *Mycobacterium tuberculosis* Infection of Multiple Animal Species in a Metropolitan Zoo

DNA Fingerprinting of *Mycobacterium tuberculosis*: Lessons Learned and Implications for the Future

Molecular Epidemiology of Tuberculosis in a Sentinel Surveillance Population

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Two Cases of Pulmonary Tuberculosis Caused by *Mycobacterium tuberculosis* subsp. *canetti*

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