

The Critical Role of Pathology in the Investigation of Bioterrorism-Related Cutaneous Anthrax

Wun-Ju Shieh,* Jeannette Guarner,*
Christopher Paddock,* Patricia Greer,*
Kathleen Tatti,* Marc Fischer,[†] Marci Layton,[‡]
Michael Phillips,[‡] Eddy Bresnitz,[§]
Conrad P. Quinn,[†] Tanja Popovic,[†]
Bradley A. Perkins,[†] Sherif R. Zaki,* and
the Anthrax Bioterrorism Investigation Team[§]

From Infectious Disease Pathology Activity,* Division of Viral and Rickettsial Diseases, and Meningitis and Special Pathogens Branch,[†] Division of Bacterial and Mycotic Diseases, National Centers for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; the Department of Health,[‡] New York City, New York; and the Department of Health and Senior Services,[§] Trenton, New Jersey

Cutaneous anthrax is a rare zoonotic disease in the United States. The clinical diagnosis traditionally has been established by conventional microbiological methods, such as culture and gram staining. However, these methods often yield negative results when patients have received antibiotics. During the bioterrorism event of 2001, we applied two novel immunohistochemical assays that can detect *Bacillus anthracis* antigens in skin biopsy samples even after prolonged antibiotic treatment. These assays provided a highly sensitive and specific method for the diagnosis of cutaneous anthrax, and were critical in the early and rapid diagnosis of 8 of 11 cases of cutaneous anthrax during the outbreak investigation. Skin biopsies were obtained from 10 of these 11 cases, and histopathological findings included various degrees of ulceration, hemorrhage, edema, coagulative necrosis, perivascular inflammation, and vasculitis. Serology was also an important investigation tool, but the results required several weeks because of the need to test paired serum specimens. Other tests, including culture, special stains, and polymerase chain reaction assay, were less valuable in the diagnosis and epidemiological investigation of these cutaneous anthrax cases. This report underscores the critical role of pathology in investigating potential bioterrorism events and in guiding epidemiological studies, a role that was clearly demonstrated in 2001 when *B. anthracis* spores were intentionally released through the United States postal system. (*Am J Pathol* 2003, 163:1901-1910)

Anthrax captured worldwide attention and aroused serious public health concerns following the intentional release of the etiological agent *Bacillus anthracis* in the United States postal system during Fall 2001,^{1,2} resulting in 11 cases of inhalational anthrax and 11 cases of cutaneous anthrax.³⁻⁷ In its conventional form, cutaneous anthrax accounts for 95% of all naturally occurring *B. anthracis* infections in the United States.⁸⁻¹¹ Patients often have a history of occupational contact with animals or animal products contaminated with *B. anthracis* spores.¹²⁻¹⁴ These pathogenic spores are introduced through a cutaneous cut or abrasion, with the most common areas of exposure are the head, neck, and extremities, although any area can be involved. Bacteremia and toxemia following cutaneous infection can occur with a fatality rate of 20% to 25% among untreated cases.¹⁵⁻¹⁷

Cutaneous anthrax is characterized by the formation of a black eschar surrounded by prominent edema and vesicles, which may resemble many other skin lesions, such as the brown recluse spider bite,¹⁸⁻²¹ ulceroglandular tularemia,^{22,23} plague,^{24,25} ecthyma gangrenosum,^{26,27} various spotted fever group rickettsial infections,²⁸⁻³¹ and scrub typhus.^{32,33} The clinical diagnosis of cutaneous anthrax is traditionally established by microbiological methods (eg, demonstrating gram-positive, capsulated bacilli on the smear of the lesion or isolating *B. anthracis* in culture).^{34,35} However, gram stain and culture for *B. anthracis* can be unrevealing for patients who receive antibiotic therapy before specimens are obtained.^{36,37} Historically, skin biopsies of cutaneous anthrax lesions have seldom been performed, and the histopathological features have been described in only a few untreated human cases and in experimental animals.³⁸⁻⁴¹

In this report, we describe the histopathological features and the immunohistochemical (IHC) findings of bioterrorism-related cutaneous anthrax cases of 2001, and compare the results of IHC assays with other laboratory diagnostic methods, including culture, special stains, polymerase chain reaction (PCR), and serology. The critical role of pathology in directing the investigation of the outbreak is also discussed.

The members of the Cutaneous Anthrax Investigation Team are: Jeanine Bartlett, Tara Ferebee-Harris, Jeltley Montague, Tim Morken, Chalanda Smith, Dan Jernigan, and David A. Ashford.

Accepted for publication July 25, 2003.

Address reprint requests to Sherif R. Zaki M.D., Ph.D., Chief, Infectious Disease Pathology Activity, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333. E-mail: szaki@cdc.gov.

Materials and Methods

From October 1 through December 31, 2001, 117 formalin-fixed skin biopsies from patients with clinically suspected cutaneous anthrax were submitted for testing to the Infectious Disease Pathology Activity (IDPA), Centers

for Disease Control and Prevention (CDC). Some clinical and demographic data accompanied each specimen, although they varied in completeness. Serum, blood, wound swab, and other clinical samples were available in some cases. Serology and reverse transcription (RT)-PCR were performed by other laboratories at CDC.⁴²⁻⁴⁴

Table 1. Demographic Information, Histopathologic Features, Special Stains and *Bacillus anthracis* Immunostaining Results

Case no./case definition and status*	Age/sex	Duration of illness/treatment† (days)	Routine histopathologic examination		<i>Bacillus anthracis</i> immunostaining
			H&E stain	Special stains	
1/Confirmed	38/F	14/9	Epidermis: mild acantholysis, mild PMN infiltrate Dermis: edema, focal necrosis, focal hemorrhage, vasculitis, intense mononuclear perivascular infiltrate	Negative	Cell wall: rare bacilliform, focal granular Capsule: focal rare granular
2/Confirmed	0.6/M	14/13	Epidermis: necrosis, hemorrhage, acantholysis, focal mild PMN infiltrate Dermis: edema, necrosis, focal mild mononuclear perivascular infiltrate	Negative	Cell wall: focal rare bacilliform and granular Capsule: widespread bacilliform and granular
3/Confirmed	27/F	15/12	Epidermis: necrosis, hemorrhage, ulceration Dermis: edema, necrosis, hemorrhage, vasculitis, intense mononuclear perivascular infiltrate	Negative	Cell wall: focal bacilliform and granular Capsule: focal bacilliform and granular
4/Confirmed	46/F	19/16	Epidermis: necrosis, hemorrhage, ulceration, PMN infiltrate Dermis: edema, necrosis, hemorrhage, vasculitis, intense focal mononuclear perivascular infiltrate	Gram stain: mixed gram-positive and gram-negative cocci Steiner's stain: mixed cocci and bacilli	Cell wall: focal abundant bacilliform and granular Capsule: focal abundant bacilliform and granular
5/Confirmed	35/M	3/1	Epidermis: necrosis, hemorrhage, acantholysis, suprabasilar cleft, ulceration, mild PMN infiltrate Dermis: edema, necrosis, hemorrhage, vasculitis, intense mixed perivascular infiltrate	Gram stain: Negative Steiner's stain: abundant bacilli in dermis	Cell wall: widespread abundant bacilliform and granular Capsule: widespread abundant bacilliform and granular
6/Suspect	34/M	5/3	Epidermis: necrosis, hemorrhage, acantholysis, bullous, intense PMN infiltrate Dermis: edema, necrosis, hemorrhage, vasculitis, intense diffuse PMN infiltrate	Gram stain: Negative Steiner's stain: focal bacilli in superficial dermis	Cell wall: widespread abundant bacilliform and granular Capsule: widespread abundant bacilliform and granular
7/Confirmed	51/F	7/6	Epidermis: necrosis, hemorrhage, ulceration, mild focal PMN infiltrate Dermis: edema, necrosis, hemorrhage, vasculitis, intense mixed perivascular infiltrate	Gram stain: a few gram-positive bacilli in epidermis and superficial dermis Steiner's stain: abundant bacilli in epidermis and superficial dermis	Cell wall: focal abundant bacilliform and granular Capsule: focal abundant bacilliform and granular
8/Confirmed	38/M	5/0	Epidermis: necrosis, suprabasillary cleft, mild PMN infiltrate, bacteria Dermis: edema, necrosis, hemorrhage, vasculitis, intense focal mononuclear perivascular infiltrate	Gram stain: abundant gram-positive bacilli in focal epidermis and superficial dermis Steiner's stain: abundant bacilli in focal epidermis and superficial dermis	Cell wall: focal abundant bacilliform, less granular Capsule: focal abundant bacilliform and granular
9/Suspect	23/F	10/8	Epidermis: necrosis, acantholysis, superficial crust, mild PMN infiltrate Dermis: edema, necrosis, hemorrhage, vasculitis, moderate mixed perivascular infiltrate	Negative	Negative‡
10/Suspect	31/F	20/13	Epidermis: small biopsy, no significant change Dermis: mild edema, mild mixed perivascular infiltrate	Negative	Negative§

*Case definition and status are described in the Methods section. One suspect case was excluded because skin biopsy was unavailable for pathologic evaluation.

†Duration of illness/antibiotic treatment prior to biopsy.

‡Patients had multiple lesions (cheek, buttock, thigh, and lower leg); the biopsy was taken from left thigh unrelated to the primary lesion in the cheek.

§Contained a small, superficial, shave biopsy that was inadequate for pathologic evaluation.

Table 2. Test Results of IHC, Culture, PCR, and Serology

Case number/status*	IHC	Culture	PCR	Serology
1/Confirmed	Positive	Negative (nasal and wound swab)	Negative (serum, swab, formalin-fixed skin biopsy)	Reactive
2/Confirmed	Positive	Negative (blood and wound swab)	Positive (one of eight blood samples) Negative (formalin-fixed skin biopsy)	NA
3/Confirmed	Positive	Negative (serum)	Negative (serum and frozen skin biopsy)	Reactive
4/Confirmed	Positive	Negative (blood)	Positive (formalin-fixed skin biopsy) Negative (blood and serum)	Reactive
5/Confirmed	Positive	Positive (blood) Negative (nasal swab and fresh skin); Staphylococcus species (wound)	Negative (frozen skin biopsy)	Reactive
6/Suspect	Positive	Negative (blood and wound swab)	Negative (blood and frozen skin biopsy)	Negative
7/Confirmed	Positive	Negative (blood, wound swab, and fresh skin)	Positive (frozen skin biopsy) Negative (serum)	Reactive
8/Confirmed	Positive	Positive (wound swab) Negative (blood and fresh skin)	Negative (serum and frozen skin biopsy)	Reactive
9/Suspect	Negative [†]	Negative (blood, serum, and nasal swab)	Negative (blood, serum, nasal swab, and formalin-fixed skin biopsy)	Reactive
10/Suspect	Negative [‡]	NA	Negative (serum and frozen skin biopsy)	Reactive

NA, not available.

*Case definition and status are described in the Methods section. One suspect case was excluded because skin biopsy was unavailable for pathologic evaluation.

[†]Patients had multiple lesions (cheek, buttock, thigh, and lower leg); the biopsy was taken from left thigh unrelated to the primary lesion in the cheek.

[‡]Contained a small, superficial, shave biopsy that was inadequate for pathologic evaluation.

All skin biopsies were examined by using hematoxylin and eosin (H&E)-stained sections for histopathological evaluation. Special stains, including gram stain and Steiner's silver stain, were used to detect bacterial agent in tissue sections. A colorimetric immunoalkaline phosphatase IHC method was developed by using two monoclonal antibodies as described below. In brief, 3- μ m sections from formalin-fixed, paraffin-embedded tissues were deparaffinized, rehydrated, and placed in a DAKO autostainer (DAKO Corporation, Carpinteria, CA). Three sections were incubated for 1 hour with a mouse monoclonal IgM antibody reactive with *B. anthracis* cell wall antigen at 1:200 dilution (USA Military Research Institute of Infectious Disease, Frederick, MD).⁴⁵ Three other sections were incubated for 1 hour with a mouse monoclonal IgM antibody reactive with *B. anthracis* capsule antigen at 1:1000 dilution. Slides to be incubated with anti-cell wall antibody were first digested in 0.1 mg/ml proteinase K (Boehringer-Mannheim Corporation, Indianapolis, IN), but slides incubated with anti-capsule antibody were not pre-digested. Optimal dilutions of the antibodies and the requirement for pre-digestion were determined by a series of pilot studies performed on positive control samples (described later). After incubation, slides were washed and incubated with a biotinylated anti-mouse IgM antibody. Antigens were visualized by using a streptavidin-alkaline phosphatase complex followed by naphthol/fast red substrate for colorimetric detection (DAKO Corporation). Sections were counterstained with Mayer's hematoxylin (Fisher Scientific, Pittsburgh, PA).

Positive controls included sections prepared from formalin-fixed, paraffin-embedded *B. anthracis* grown in culture and tissues obtained from confirmed animal and fatal human anthrax cases.⁴⁶ Negative controls consisted of the following: 1) each patient's tissue sections incubated with an IgM antibody reactive with an irrelevant infectious



Figure 1. Histopathology and immunohistochemical staining on cutaneous anthrax index case (case 1). This patient received antibiotics treatment for 9 days before the biopsy was taken. **A:** Skin biopsy from the edge of eschar showed intact epidermis; the dermis showed edema, focal hemorrhage, and perivascular mononuclear cell infiltrates. Superficial ulceration and fibrinopurulent membrane typically associated with acute lesions were not seen. **B:** Focal, rare immunohistochemical staining of *B. anthracis* cell wall antigen was present in the dermis. H&E stain (**A**); immunalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain (**B**). Original magnifications: $\times 25$ (**A**); $\times 250$ (**B**).

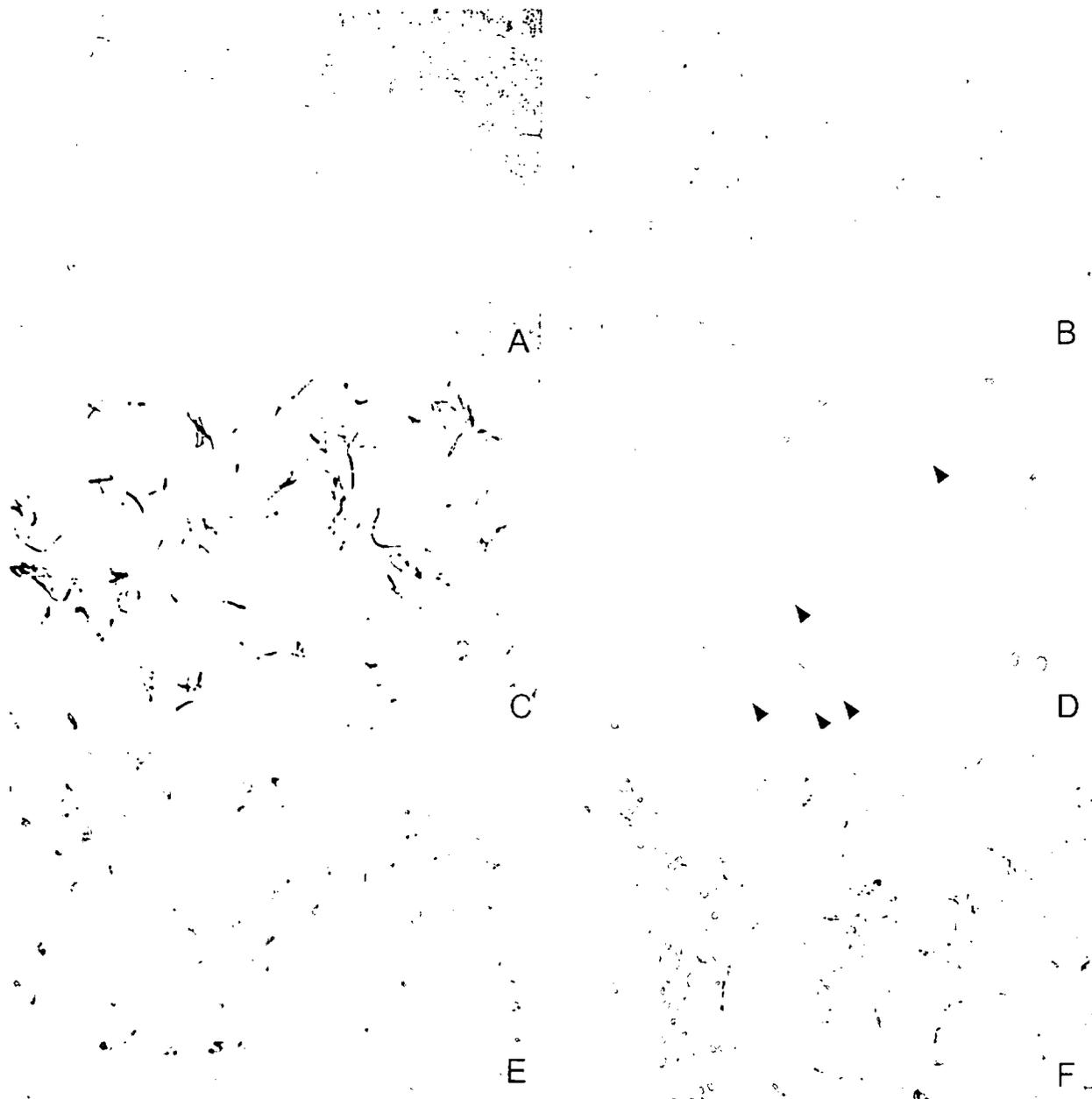


Figure 2. Histopathology, special stains, and immunohistochemical staining on case 7. This patient received antibiotics treatment for 6 days before the biopsy was taken. **A:** Skin biopsy showed superficial ulceration, dermal edema, necrosis, and hemorrhage. **B:** Higher-power magnification showed a mixed inflammatory infiltrate in the dermis. **C:** Steiner's silver stain showed many bacilli in the dermis. **D:** Gram stain in the same area showed only a few gram-positive bacilli (arrowheads). **E:** Abundant immunohistochemical staining of granular and bacilliform antigens were demonstrated in the dermis by using anti-*B. anthracis* cell wall antibody. **F:** Immunohistochemical staining of *B. anthracis* capsule antigens was present in fibrohistiocytic cells. H&E stain, **A** and **B**; Steiner's silver stain, **C**; gram stain, **D**; immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, **E** and **F**. Original magnifications: $\times 12.5$ (**A**); $\times 100$ (**B** and **F**); $\times 250$ (**C** and **D**); $\times 158$ (**E**).

agent, 2) cutaneous lesions caused by non-anthrax etiologies, including human herpesvirus 1 (*Herpes simplex* 1), human herpesvirus 3 (Varicella-zoster virus), human herpesvirus 5 (Cytomegalovirus), *Fransicella tularensis*,

spotted fever group rickettsiae, and spider bite, and 3) formalin-fixed, paraffin-embedded *Bacillus subtilis*, *Bacillus cereus*, *Clostridium novyi*, *Clostridium sordelli*, *Streptococcus pneumoniae*, Group A *Streptococcus*, *Staphylococ-*

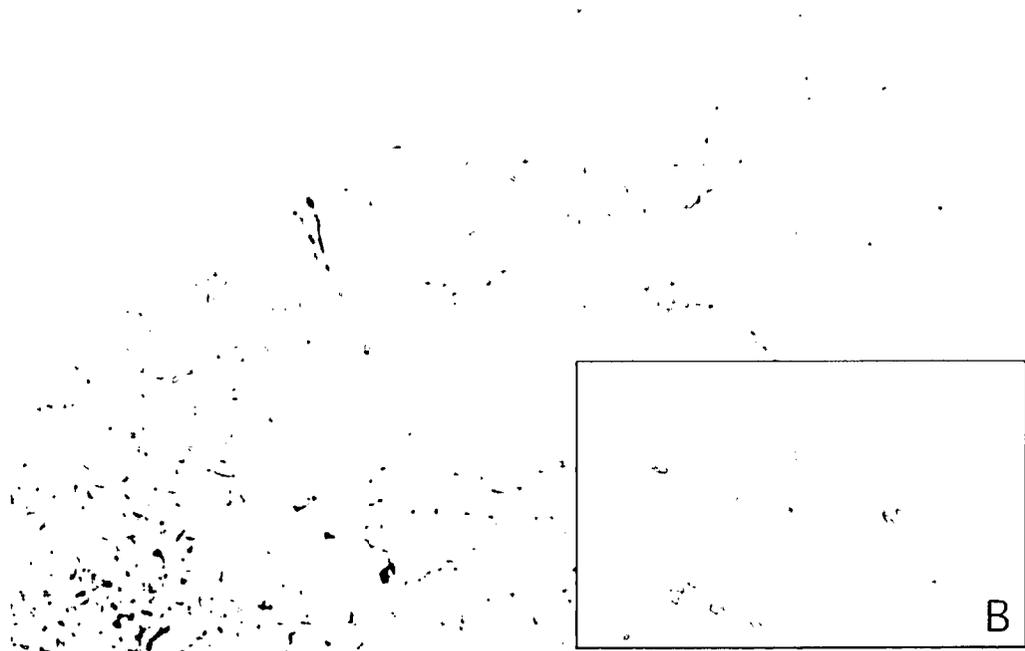
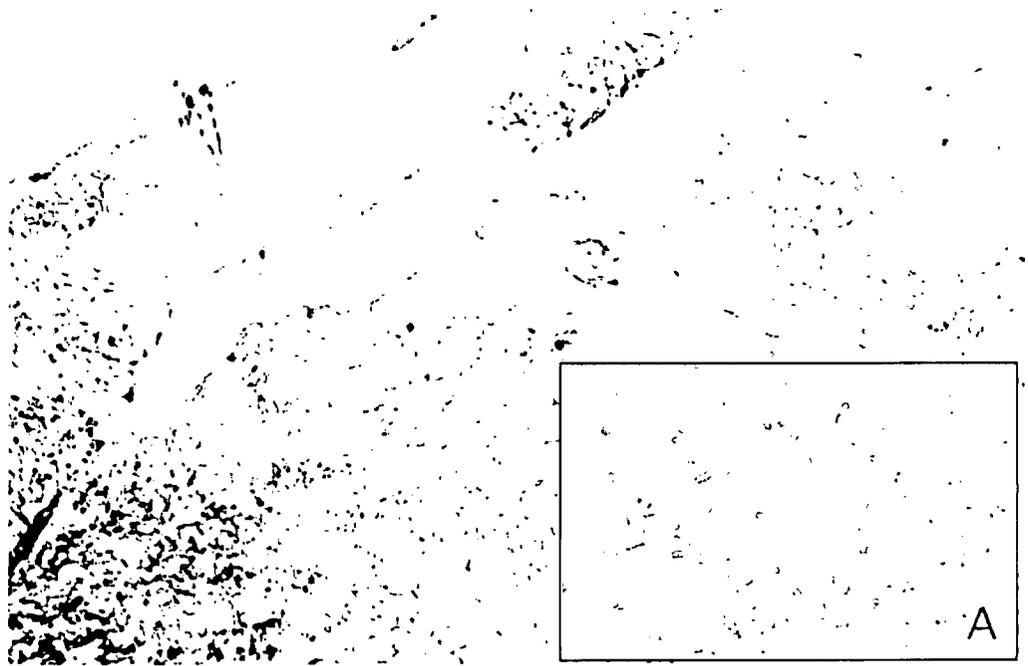
Figure 3. Histopathology and immunohistochemical staining on case 6. This patient received antibiotics treatment for 3 days before the biopsy was taken. **A:** Skin biopsy showed acantholysis, diffuse polymorphonuclear cell infiltrate, dermal edema, necrosis, and hemorrhage. **B:** Immunohistochemical staining showed focal bacilliform antigen staining by anti-*B. anthracis* cell wall antibody in the same area as **A**. **C:** Vasculitis and intense polymorphonuclear cell infiltrate were present in the dermis. **D:** Immunohistochemical staining of granular and bacilliform antigens were demonstrated by using anti-*B. anthracis* capsule antibody in the same area as **C**. H&E stain, **A** and **C**; immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, **B** and **D**. Original magnifications: $\times 100$ (**A** and **C**); $\times 250$ (**B**); $\times 158$ (**D**).

A

B

C

D



C

D

cus aureus, *Staphylococcus epidermidis*, *Pseudomonas*, *Klebsiella pneumoniae*, *Coccidioides*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*.

A confirmed case of cutaneous anthrax was defined as a clinically compatible case that was either laboratory confirmed by isolation of *B. anthracis* from an affected tissue or site, or accompanied with other laboratory evidence of *B. anthracis* infection based on at least two supportive laboratory tests, including 1) evidence of *B. anthracis* DNA by PCR from specimens collected from an affected tissue or site, 2) demonstration of *B. anthracis* in a clinical specimen by IHC, or 3) four-fold rise in anti-protective antigen (PA) IgG. A suspect case of cutaneous 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 epidemiological link to an environmental *B. anthracis* exposure.^{6,7,47}

Results

During the bioterrorism event of 2001, skin biopsy samples of 117 cases were tested at IDPA and 8 were positive for *B. anthracis* by IHC. There were two cases with detectable levels of serum antibody to anthrax toxin PA, but IHC and other laboratory tests were negative. Selected demographic information, histopathological features, laboratory test results, and case status for the 8 IHC-positive cases and the 2 IHC-negative, serology-reactive cases are summarized in Table 1 and Table 2. Based on the case definition for cutaneous anthrax, there were 7 confirmed cases: 2 based on culture and other diagnostic tests, including IHC (numbers 5 and 8); 2 based on IHC and serology (numbers 1 and 3); 2 based on all three supportive tests: IHC, serology and PCR (numbers 4 and 7); and 1 based on IHC and PCR (number 2). There were 3 suspect cases: 1 based only on IHC (number 6); 2 based only on serology (numbers 9 and 10). One suspect case, described in another report,⁷ was excluded because no skin biopsy sample was available for histopathological evaluation and IHC testing.

Microscopic examination of the epidermis of these 10 biopsy specimens showed 8 with necrosis, 6 with hemorrhage, 6 with acantholysis or bullous formation, and 4 with various degrees of epidermal ulceration (Figure 1, Figure 2, and Figure 3). Examination of the dermis showed various degrees of edema in all 10 specimens, coagulation necrosis in 9, and hemorrhage and vasculitis in 8 each (Figures 1 to 3). Various degrees of inflammation were present in the lower epidermis and dermis of all case specimens (Figure 1A, Figure 2B, and Figure 3, A and C). Polymorphonuclear infiltrates were often present

with prominent spongiosis in the lower epidermis (Figure 3A). The dermis was usually markedly edematous, with separation of collagen bundles, and often contained an intense perivascular inflammatory infiltrate (Figure 1A and Figure 3C). Inflammatory changes were not consistent among cases, and varied from mixed to predominantly mononuclear (Table 1).

Bacilli morphologically compatible with *B. anthracis* were detected by routine H&E stain in only 1 case (number 8) (Figure 4C). Gram staining of tissue demonstrated unequivocal gram-positive bacilli in 2 cases (numbers 7 and 8) (Figure 2D and Figure 4D), and Steiner's silver stain showed bacilli compatible with *B. anthracis* in 4 cases (numbers 5, 6, 7, and 8) (Figure 2C). Case number 4 showed mixed cocci and bacilli in the biopsy samples and was difficult to evaluate. IHC staining revealed *B. anthracis* antigens in 8 cases, and all of them were positive by using both anti-cell wall and anti-capsule antibody (Table 1). Extracellular bacilliform and granular antigens were distributed in both epidermis and dermis (Figures 1 to 4). Intracellular immunostaining was also observed in fibrohistiocytic cells, and usually more prominent by anti-capsule antibody (Figure 3D). The amount and distribution of bacterial antigens among cases varied from rare (Figure 1B and Figure 3B) to abundant (Figure 2E and Figure 4A) and from focal to widespread (Table 1). Of the two skin biopsies with negative IHC results (numbers 9 and 10), one was obtained from a skin lesion unrelated to the primary lesion, and the other was an extremely small, superficial, shave biopsy that was inadequate for pathological evaluation.

Discussion

Since 1950, human anthrax in the United States was confined to those occupationally at risk and its incidence has been very low owing to improvements in animal husbandry and the handling of animal products.^{48,49} There were 235 confirmed cases reported from 1955 to 2002, and most of them were cutaneous anthrax.^{8,10} Before autumn 2001, all cases of cutaneous anthrax in the United States were related to agricultural or industrial exposures. However, the epidemiology and laboratory diagnosis of cutaneous anthrax changed after October 2001 because of the intentional release of *B. anthracis* spores through the U.S. postal system and the subsequent identification of anthrax in 22 patients, including 11 with cutaneous disease.^{6,7}

The index cutaneous anthrax case-patient from the 2001 outbreak, a 38-year-old woman who worked at a major news network, initially developed an ulcerative lesion on the chest and was seen by an infectious disease clinician. She reported handling "threat" letters at work.

Figure 4. Histopathology, special stain, and immunohistochemical staining on case 8. This patient did not receive antibiotics treatment before the biopsy was taken. **A:** Abundant immunohistochemical staining of *B. anthracis* capsule antigens was present extracellularly and intracellularly. **Inset:** Higher-power magnification showed bacilliform and granular antigens. **B:** Immunohistochemical staining of *B. anthracis* cell wall antigens was present focally. The amount of immunostaining is less than capsule antigens in **A**. **Inset:** Higher-power magnification showed bacilliform and granular antigens. **C:** H&E stain showed encapsulated bacilli in the dermis. This is the only case in which morphologically compatible bacilli were discernable by routine H&E stain. **D:** Gram stain demonstrated abundant gram-positive bacilli in the same area as **C**. Immunalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain, **A** and **B**. H&E stain. **C:** gram stain. **D:** Original magnifications: $\times 50$ (**A** and **B**); $\times 250$ (**A**, **inset**; **B**, **inset**; **C** and **D**).

Bacterial cultures of specimens both from the lesion and from a suspicious letter (dated September 25) tested negative for *B. anthracis*, and the patient was administered ciprofloxacin.

After learning of a patient with inhalational anthrax in Florida,^{4,6} the patient contacted the New York City Department of Health, and a skin biopsy was obtained 9 days after antibiotic treatment began. The biopsy was negative for *B. anthracis* by PCR, and gram stain did not reveal any bacteria. However, a rapid IHC test for *B. anthracis* showed focal granular and rare bacilliform antigen staining in the dermis (Figure 1B) establishing the diagnosis of cutaneous anthrax.

Identification of this index cutaneous anthrax case prompted a soaring public health concern with ensuing actions, such as mobilization of multidisciplinary teams for onsite investigations and initiation of antibiotic distribution at clinics. The diagnosis also led to the identification of another threat letter (dated September 18) containing *B. anthracis* spores. In the following days, several other possible cases of anthrax were reported at other major media outlets, and in most of them the diagnosis was established by IHC.^{4,5} The epidemiology of anthrax in this outbreak was shifted from the traditional occupational hazard of zoonotic contact to a deliberate bioterroristic action involving the U.S. postal system.⁵⁰

Cutaneous anthrax lesions can be difficult to diagnose clinically and histopathologically. During investigation of the cutaneous anthrax cases, skin lesions were frequently mistaken for other diseases, including those caused by human herpes simplex virus, *Varicella zoster* virus, rickettsial pox, and arthropod bite. These diseases were diagnosed by IHC for specific agents and by histopathological evaluation as well. In this report, we found that histopathological features of cutaneous anthrax lesions can vary with the duration of illness, modality of treatment, and site of biopsy in relation to the lesion. In general, perivascular infiltrates are predominantly mononuclear in cases with longer duration of illness and treatment course, while polymorphonuclear infiltrates are more prominent in cases with shorter duration (Table 1). Biopsies obtained from the edge of an eschar did not show superficial ulceration with fibrinopurulent membrane typically associated with biopsies taken from the center of eschar of cutaneous anthrax.^{38,41}

Laboratory evidence was critical in the diagnosis of suspect cutaneous anthrax cases during investigation of this outbreak. Traditionally, cutaneous anthrax was diagnosed by microbiological methods and skin biopsy was seldom done for histopathological examination. Although culture and gram stain of specimens from suspicious skin lesions remain essential methods for diagnosing cutaneous anthrax, their sensitivity decreases significantly when the patient has received antibiotic treatment before specimen collection. In general, the longer the duration of illness and treatment course, the less chance of detecting *B. anthracis* organism by culture and special stains. The organism is best demonstrated when the lesion is in the vesicular stage, so swab exudates for gram stain and culture should be performed before initiation of antibiotic therapy. Similar observation in histopathological evalua-

tion and tissue special stains in this study is shown in Table 1. The only case showing encapsulated bacilli in the skin by H&E (number 8, Figure 4C) was also the only case that skin biopsy was taken before initiation of antibiotic treatment. The 4 cases (numbers 5, 6, 7, and 8) showing bacilli morphologically compatible with *B. anthracis* by special stains were those with skin biopsies taken within 1 week of antibiotic treatment. In these cases, Steiner's silver stain was more useful than gram stain for demonstrating these bacilli in the cutaneous lesion. Because IHC testing of skin biopsies can detect bacterial antigens in tissues regardless of the treatment, it provides a more sensitive and specific way to establish the diagnosis of cutaneous anthrax. Using two monoclonal antibodies against different epitopes of *B. anthracis* further enhances the sensitivity and specificity of IHC assays.

Other laboratory methods, such as serology and PCR, are also important investigation tools. Serology is very useful for surveillance and diagnosis of cutaneous anthrax, but the results require several weeks because of the need to test paired serum specimens. PCR is a sensitive and specific method to detect bacterial DNA in clinical samples,^{43,51-53} but it does not provide a morphological correlation with the result. Moreover, even real-time PCR is not as sensitive as IHC in this study for *B. anthracis* detection, probably because of the treatment issues.

The following recommendations for obtaining skin biopsies were proposed based on a combination of histopathological findings and localization of bacterial antigens: 1) a full-thickness punch biopsy fixed in 10% buffered formalin from a papule or vesicle lesion and including adjacent skin, and 2) biopsies should be taken from both vesicle and eschar, if present. All biopsy specimens should be accompanied by pertinent clinical information, including a brief history, description, and chronology of the lesion(s); specific treatment; and date of biopsy in relation to antibiotic treatment. A photograph, digital image, or diagram indicating the site of each biopsy in relation to the lesion would be particularly helpful. The above recommendations were adapted by the American Academy of Dermatology and published as part of the management algorithm.⁵⁴ Based on the study results described in this report, a skin biopsy should be obtained whenever cutaneous anthrax is suspected, preferably before initiation of antibiotic treatment, and evaluated by IHC testing. Although PCR is usually performed on fresh tissue, it can be done on formalin-fixed tissue as well. Dividing a single biopsy specimen for multiple tests is not recommended because of the focal nature of immunostaining.

Pathology has been a critical component in a multidisciplinary team for detection, surveillance, and research of emerging and reemerging pathogens.⁵⁵⁻⁵⁹ With the increasing concern of bioterrorism, pathologists are now playing an even more prominent role in both responding to a known terrorist event and in conducting surveillance for unusual deaths or clusters of critical illness that may represent unannounced terrorist activity. The investigation of both inhalational and cutaneous anthrax cases

clearly exemplifies these roles.^{6,7,60,61} In addition to *B. anthracis*, several rapid and sensitive IHC assays have been developed for detecting other bioterrorism-related agents, such as *Ebola virus*,^{62,63} *Fransicella tularensis*,⁶⁴ *Yersinia pestis*,⁶⁵ and smallpox virus. These assays provide a method for rapid diagnosis and have fortified the preparedness of responses to possible bioterrorism events.

References

1. Lane HC, Fauci AS: Bioterrorism on the home front: a new challenge for American medicine. *JAMA* 2001, 286:2595-2597
2. Brookmeyer R, Blades N: Prevention of inhalational anthrax in the U. S. outbreak. *Science* 2002, 295:1861
3. CDC: Recognition of illness associated with the intentional release of a biologic agent. *MMWR Morb Mortal Wkly Rep* 2001, 50:893-897
4. CDC Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. *MMWR Morb Mortal Wkly Rep* 2001, 50:889-893
5. CDC Update: investigation of bioterrorism-related anthrax, 2001. *MMWR Morb Mortal Wkly Rep* 2001, 50:1008-1010
6. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, Tapper M, Fisk TL, Zaki S, Popovic T, Meyer RF, Quinn CP, Harper SA, Fridkin SK, Sejvar JJ, Shepard CW, McConnell M, Guarner J, Shieh WJ, Malecki JM, Gerberding JL, Hughes JM, Perkins BA: Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg Infect Dis* 2001, 7:933-944
7. Jernigan DB, Raghunathan PL, Bell BP, Brechner R, Bresnitz EA, Butler JC, Cetron M, Cohen M, Doyle T, Fischer M, Greene C, Griffith KS, Guarner J, Hadler JL, Hayslett JA, Meyer R, Petersen LR, Phillips M, Pinner R, Popovic T, Quinn CP, Reefhuis J, Reisman D, Rosenstein N, Schuchat A, Shieh WJ, Siegal L, Swerdlow DL, Tenover FC, Traeger M, Ward JW, Weisfuse I, Wiersma S, Yeskey K, Zaki S, Ashford DA, Perkins BA, Ostroff S, Hughes J, Fleming D, Koplan JP, Gerberding JL: Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. *Emerg Infect Dis* 2002, 8:1019-1028
8. CDC: Human anthrax associated with an epizootic among livestock: North Dakota, 2000. *MMWR Morb Mortal Wkly Rep* 2001, 50:677-680
9. Taylor JP, Dimmitt DC, Ezzell JW, Whitford H: Indigenous human cutaneous anthrax in Texas. *South Med J* 1993, 86:1-4
10. CDC: Human cutaneous anthrax: North Carolina, 1987. *MMWR Morb Mortal Wkly Rep* 1988, 37:413-414
11. Paulshock BZ: When anthrax was a Delaware Valley disease: Dr. Herman Gold's experience with cutaneous anthrax. *Del Med J* 2001, 73:159-162
12. Steele JH: Occupational health in agriculture: animal-borne diseases. *Arch Environ Health* 1968, 17:267-285
13. Wilson A: Animal health and the E.E.C.: notifiable diseases in Great Britain and the E.E.C countries. *Br Vet J* 1972, 128:226-234
14. Smith IM: A brief review of anthrax in domestic animals. *Postgrad Med J* 1973, 49:571-572
15. LaForce FM: Anthrax. *Clin Infect Dis* 1994, 19:1009-1013; quiz 1014
16. Gold H: Anthrax: a report of 117 cases. *Arch Int Med* 1955, 96:387
17. McSwiggan DA, Hussain KK, Taylor IO: A fatal case of cutaneous anthrax. *J Hyg (Lond)* 1974, 73:151-156
18. Mallon E, McKee PH: Extraordinary case report: cutaneous anthrax. *Am J Dermatopathol* 1997, 19:79-82
19. Freedman A, Afonja O, Chang MW, Mostashari F, Biaser M, Perez-Perez G, Lazarus H, Schacht R, Guttenberg J, Traister M, Borkowsky W: Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a 7-month-old infant. *JAMA* 2002, 287:869-874
20. Elston DM, Eggers JS, Schmidt WE, Storrow AB, Doe RH, McGlasson D, Fischer JR: Histological findings after brown recluse spider envenomation. *Am J Dermatopathol* 2000, 22:242-246
21. Wilson DC, King LE Jr: Spiders and spider bites. *Dermatol Clin* 1990, 8:277-286
22. Senol M, Ozcan A, Karıncaoglu Y, Aydin A, Ozerol IH: Tularemia: a case transmitted from a sheep. *Cutis* 1999, 63:49-51
23. Kodama BF, Fitzpatrick JE, Gentry RH: Tularemia. *Cutis* 1994, 54:279-280
24. Merchant SR, Taboada J: Systemic diseases with cutaneous manifestations. *Vet Clin North Am Small Anim Pract* 1995, 25:945-959
25. Butler T: A clinical study of bubonic plague: observations of the 1970 Vietnam epidemic with emphasis on coagulation studies, skin histology and electrocardiograms. *Am J Med* 1972, 53:268-276
26. Gucluer H, Ergun T, Demircay Z: Ecthyma gangrenosum. *Int J Dermatol* 1999, 38:299-302
27. Greene SL, Su WP, Muller SA: Ecthyma gangrenosum: report of clinical, histopathologic, and bacteriologic aspects of eight cases. *J Am Acad Dermatol* 1984, 11:781-787
28. Kao GF, Evancho CD, Ioffe O, Lowitt MH, Dumler JS: Cutaneous histopathology of Rocky Mountain spotted fever. *J Cutan Pathol* 1997, 24:604-610
29. Kass EM, Szaniawski WK, Levy H, Leach J, Srinivasan K, Rives C: Rickettsialpox in a New York City hospital, 1980 to 1989. *N Engl J Med* 1994, 331:1612-1617
30. Dujella J, Morovic M, Dzelalija B, Gveric M, Novakovic S: Histopathology and immunopathology of skin biopsy specimens in Mediterranean spotted fever. *Acta Virol* 1991, 35:566-572
31. Walker DH, Occhino C, Tringali GR, Di Rosa S, Mansueto S: Pathogenesis of rickettsial eschars: the tache noire of boutonneuse fever. *Hum Pathol* 1988, 19:1449-1454
32. Choi YH, Kim SJ, Lee JY, Pai HJ, Lee KY, Lee YS: Scrub typhus: radiological and clinical findings. *Clin Radiol* 2000, 55:140-144
33. Fang RC, Lin WP, Chao PS, Kuo NT, Chen CM: Clinical observations of scrub typhus on Penghu (the Pescadore Islands). *Trop Geogr Med* 1975, 27:143-150
34. Caksen H, Arabaci F, Abuhandan M, Tuncer O, Cesur Y: Cutaneous anthrax in eastern Turkey. *Cutis* 2001, 67:488-492
35. Shlyakhov EN, Shvarts SA, Gruz EV: Anthrax: Biological and immunological principles of diagnosis and prevention. Communication 7. Diagnosis of anthrax by means of anthraxin. *J Hyg Epidemiol Microbiol Immunol* 1973, 17:279-284
36. 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, 15:242-245
37. Shlyakhov EN, Gruz EV, Burdenko TA: Anthrax: biological and immunological principles of diagnosis and prevention. 6. The effect of specific treatment on the diagnosis of anthrax using anthraxin. *J Hyg Epidemiol Microbiol Immunol* 1973, 17:85-88
38. Lebowich RJ, McKillip BG, Conboy JR: Cutaneous Anthrax: a pathological study with clinical correlation. *Am J Clin Pathol* 1943, 13:505-515
39. Berdjis CC, Gleiser CA: Experimental subcutaneous anthrax in chimpanzees. *Exp Mol Pathol* 1964, 3:63
40. Dutz W, Kohout E: Anthrax. *Pathol Annu* 1971, 6:209-248
41. Zaucha GM, Pitt LM, Estep J, Ivins BE, Friedlander AM: The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous inoculation. *Arch Pathol Lab Med* 1998, 122:982-992
42. De BK, Bragg SL, Sanden GN, Wilson KE, Diem LA, Marston CK, Hoffmaster AR, Barnett GA, Weyant RS, Abshire TG, Ezzell JW, Popovic T: A two-component direct fluorescent-antibody assay for rapid identification of *Bacillus anthracis*. *Emerg Infect Dis* 2002, 8:1060-1065
43. Sacchi CT, Whitney AM, Mayer LW, Morey R, Steigerwalt A, Boras A, Weyant RS, Popovic T: Sequencing of 16S rRNA gene: a rapid tool for identification of *Bacillus anthracis*. *Emerg Infect Dis* 2002, 8:1117-1123
44. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, Stamey K, Steward-Clark E, Schmidt DS, Mothershed E, Pruckler J, Schwartz S, Benson RF, Heisel LO, Holder PF, Johnson SE, Keilum M, Messmer T, Thacker WL, Besser L, Plikaytis BD, Taylor TH, Jr., Freeman AE, Wallace KJ, Dull P, Sejvar J, Bruce E, Moreno R, Schuchat A, Lingappa JR, Martin SK, Walls J, Bronsdon M, Carlone GM, Bajani-Ari M, Ashford DA, Stephens DS, Perkins BA: Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. *Emerg Infect Dis* 2002, 8:1103-1110
45. Ezzell JW, Jr., Abshire TG, Little SF, Lidgerding BC, Brown C: Identification of *Bacillus anthracis* by using monoclonal antibody to cell wall galactose-N-acetylglucosamine polysaccharide. *J Clin Microbiol* 1990, 28:223-231

46. Ringertz SH, Hoiby EA, Jensenius M, Maehlen J, Caugant DA, Myklebust A, Fossum K: Injectional anthrax in a heroin skin-popper. *Lancet* 2000, 356:1574-1575
47. CDC Update: investigation of bioterrorism-related anthrax and adverse events from antimicrobial prophylaxis. *MMWR Morb Mortal Wkly Rep* 2001, 50:973-976
48. Hugh-Jones M: 1996-97 Global Anthrax Report. *J Appl Microbiol* 1999, 87:189-191
49. Anthrax: memorandum from a WHO meeting. *Bull World Health Organ* 1996, 74:465-470
50. Sidel V, Cohen HW, Gould RM: From woolsorters to mail sorters: anthrax past, present, and future. *Am J Public Health* 2002, 92:705-706
51. 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, 8:1111-1116
52. Ellerbrok H, Nattermann H, Ozel M, Beutin L, Appel B, Pauli G: Rapid and sensitive identification of pathogenic and apathogenic *Bacillus anthracis* by real-time PCR. *FEMS Microbiol Lett* 2002, 214:51
53. Jackson PJ, Hugh-Jones ME, Adair DM, Green G, Hill KK, Kuske CR, Grinberg LM, Abramova FA, Keim P: 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 USA* 1998, 95:1224-1229
54. Carucci JA, McGovern TW, Norton SA, Daniel CR, Elewski BE, Fallon-Friedlander S, Lushniak BD, Taylor JS, Warschaw K, Wheeland RG: Cutaneous anthrax management algorithm. *J Am Acad Dermatol* 2001, 21:21
55. Abramova FA, Grinberg LM, Yampolskaya OV, Walker DH: Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979. *Proc Natl Acad Sci USA* 1993, 90:2291-2294
56. Zaki SR, Greer PW, Colfield LM, Goldsmith CS, Nolte KB, Foucar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS, Rollin PE, Ksiazek TG, Nichol ST, Mahy BWJ, Peters CJ: Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. *Am J Pathol* 1995, 146:552-579
57. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG: A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996, 347:921-925
58. Shieh WJ, Guarner J, Layton M, Fine A, Miller J, Nash D, Campbell GL, Roehrig JT, Gubler DJ, Zaki SR: The role of pathology in an investigation of an outbreak of West Nile encephalitis in New York, 1999. *Emerg Infect Dis* 2000, 6:370-372
59. Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE, Ksiazek TG, Zaki SR: Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol* 2002, 161:2153-2167
60. Barakat LA, Quentzel HL, Jernigan JA, Kirschke DL, Griffith K, Spear SM, Kelley K, Barden D, Mayo D, Stephens DS, Popovic T, Marston C, Zaki SR, Guarner J, Shieh WJ, Carver HW II, Meyer RF, Swerdlow DL, Mast EE, Hadler JL: Fatal inhalational anthrax in a 94-year-old Connecticut woman. *JAMA* 2002, 287:863-868
61. Guarner J, Jernigan JA, Shieh WJ, Tatti K, Flannagan LM, Stephens DS, Popovic T, Ashford DA, Perkins BA, Zaki SR, Inhalational Anthrax Pathology Working Group: Pathology and pathogenesis of bioterrorism-related inhalational anthrax. *Am J Pathol* 2003, 163:701-709
62. Zaki SR, Shieh WJ, Greer PW, Goldsmith CS, Ferebee T, Katshitshi J, Tshioko FK, Bwaka MA, Swanepoel R, Calain P, Khan AS, Lloyd E, Rollin PE, Ksiazek TG, Peters CJ: A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. *Commission de Lutte contre les Epidemies a Kikwit. J Infect Dis* 1999, 179(Suppl 1):S36-S47
63. Zaki SR, Peters LJ: Viral hemorrhagic fevers. Pathology of Infectious Diseases. Edited by DH Connor, FW Chandler, DA Schwartz, HJ Manz, EE Lack. Stamford CT, Appleton and Lange, 1997. pp 347-364
64. Guarner J, Greer PW, Bartlett J, Chu MC, Shieh WJ, Zaki SR: Immunohistochemical detection of *Francisella tularensis* in formalin-fixed paraffin-embedded tissue. *Appl Immunohisto Mol Morphol* 1999, 7:122-126
65. Guarner J, Shieh WJ, Greer PW, Gabastou JM, Chu M, Hayes E, Nolte KB, Zaki SR: Immunohistochemical detection of *Yersinia pestis* in formalin-fixed, paraffin-embedded tissue. *Am J Clin Pathol* 2002, 117:205-209