



Anthrax September 2003

Anthrax is an ancient zoonotic disease primarily affecting herbivorous animals that is caused by the bacterium *Bacillus anthracis*. Of all of the potential pathogens we would expect to encounter as a bioterrorism agent, we have the most knowledge about the use of *B. anthracis* for this purpose.

Our understanding of anthrax and its use as a biological weapon stems primarily from information gained through the epidemiologic investigations of inhalational anthrax outbreaks from three sources: the 18 cases in the United States from 1900-1976, associated with various occupational exposures; the inadvertent aerosolization of anthrax spores being produced for weapons purposes at a plant in Sverdlovsk, Russia in 1979; and most recently, we have substantial information providing new insights into the presentation and pathogenesis of anthrax infection caused by anthrax spores in powder form that was delivered in letters during the U.S. outbreak of 2001, the first known successful deliberate use of anthrax as an agent of terror.

In the 2001 letter-associated outbreak there were 22 cases of anthrax in multiple Eastern states. Of the 22, 10 were inhalational and 12 were cutaneous. All 5 of the fatal cases were of the inhalational form. Although details of the Sverdlovsk outbreak are limited because of the secrecy surrounding the episode, there were at least 77 human cases and 66 deaths, all of which were downwind of the plant. Many were found to be infected with more than one strain, raising fears that multiple strains with differing antibiotic susceptibilities, including genetically altered resistance patterns, could be encountered from a single intentional release.

There are 3 recognized forms of human anthrax, determined by where spores germinate. Inhalational anthrax, which is the most likely form to be seen in a bioterrorism event, is rare in naturally-occurring disease, comprising less than 5% of all cases. However, the mortality is high, ranging from 45% in the 2001 cases to 89% in 20th century U.S. cases. It is not clear why there is such a discrepancy in mortality between current and past cases, but possible factors include improved antibiotic and critical care facilities, strain

characteristics or inoculum dose. Cutaneous disease is the most common form of natural disease comprising 95% of all cases. Mortality is less than 1% in treated cases, but up to 20% in cases that are left untreated. Gastrointestinal disease is rare, comprising less than 5% of all cases worldwide and has never been reported in the United States. Mortality numbers are not well known, however, it is estimated to be at least 50%.

The key to anthrax infection is that there must be contact with spores, either through natural or intentional circumstances. Traditional risk factors for naturally-occurring inhalational and cutaneous disease are similar, namely contact with the hides and skins of infected animals. Gastrointestinal infection has occurred by ingestion of meat from infected animals. It has been known for decades that anthrax spores could be aerosolized, thus providing a risk for large scale human exposure. However, the investigations from the 2001 outbreak identified a new risk factor for anthrax that had not been considered in the past: powdered material containing anthrax spores being sent through the mail. Exposure to the spores by handling the contaminated letters led to several cases of cutaneous disease. Also, because the powder was milled into fine particles of 5 microns or less, spores were presumably aerosolized upon mechanical processing of the letters in postal facilities and upon handling of the opened letters, leading to at least 9 of the inhalational anthrax cases. Exposures for the remaining two inhalational cases were hypothesized to be cross-contamination of the mail, but there was insufficient evidence to confirm this.

Bacillus anthracis is the bacterium that causes anthrax. In its vegetative, metabolically active form it is a large, aerobic encapsulated Gram-positive rod, ranging in size from 3 to 10 microns long and usually 1 micron in width. The organisms are non-motile and non-hemolytic on standard blood agar. Long chains are formed in vitro, while short chains or single bacteria are usually seen in direct examination of clinical specimens, sometimes displaying a “jointed bamboo rod” appearance. Gram-positive bacilli in clinical specimens are often considered contaminants by microbiology laboratories, as most other Gram positive bacilli such as *Diphtheroides* and *Corynebacteria* species are commonly isolated non-pathogenic skin flora. If anthrax is suspected, the laboratory should be alerted to ensure full workup of any Gram positive rod. *Bacillus anthracis* is a sporulating organism, thus when exposed to oxygen or a nutrient-depleted milieu, it converts to a hardy inert, but infectious, spore one micron in size that can survive for years, even under harsh conditions. When spores land in a nutrient-rich environment, they germinate into the vegetative bacillus state and begin producing the toxins that lead to disease.

This slide demonstrates the “jointed bamboo rod” appearance of Gram positive staining *Bacillus anthracis* in a specimen of CSF from the index case of the 2001 outbreak. Note the short chains and large size.

This diagram of anthrax pathogenesis nicely illustrates the common processes that occur for all three forms of anthrax. Regardless of exposure route, macrophages engulf the spores where they land. Inhaled spores less than 5 microns can travel to the alveoli, while larger particles may land in the oropharynx and cause GI disease. Macrophages

then carry the spores to regional lymph nodes, except in most cases of cutaneous disease where the infection remains localized in the skin. Within this nutrient-rich environment, the spores germinate into vegetative bacilli. Rapid multiplication results in lysis of macrophages and destruction of local lymph node or subcutaneous architecture. This is the mechanism for the pathognomonic hemorrhagic mediastinal lymphadenitis of inhalational disease. Of note, because the spores do not germinate in the alveoli, a true pneumonia rarely, if ever, develops in inhalational anthrax. During bacterial replication, high levels of the two toxins are produced. Edema toxin causes massive edema at the site of germination in cutaneous disease and large pleural effusions in inhalational. Lethal toxin initiates the cascade of inflammatory mediators that leads to sepsis when systemic disease occurs. High levels of toxin in the blood inevitably lead to death even if the bacteria are quickly killed with antibiotics.

In most cases of inhalational and GI disease, and some untreated cutaneous cases, high-grade bacteremia with subsequent systemic illness and sepsis occur rapidly. The degree of bacteremia is much higher than most other bacterial infections, and the bacterial burden can be so great that direct Gram stains of peripheral blood buffy coats, such as the one in this photomicrograph, often demonstrate the Gram positive bacilli.

The clinical features of inhalational anthrax have been fairly well described in the past, and have been further validated by the 2001 outbreak. An asymptomatic incubation period occurs, which can range between 2 and 43 days, generally lasting 4-7 days from exposure to symptom onset. The occasional longer incubation periods are thought to be related to delayed spore germination, which, in animal studies occurred up to 98 days after exposure. Symptoms classically follow a biphasic pattern, starting with a prodrome consisting of a nonspecific flu-like syndrome then progressing to a rapidly fatal fulminant phase. Early symptoms are nearly always constitutional (fevers, malaise, myalgias) and respiratory (dyspnea, nonproductive cough, chest discomfort) and often are gastrointestinal (nausea, vomiting, diarrhea). Notably absent in most cases are nasal symptoms such as rhinorrhea and congestion. This feature may help to differentiate inhalational anthrax in the prodromal stage from upper respiratory viral illnesses. The duration of the prodromal phase is typically several hours to 3 days. It is important to also realize that a flu-like prodrome occurs in many of the other bioterrorism-related diseases. Disease then progresses to a fulminant phase, where patients are critically ill with fever, diaphoresis, respiratory distress and cyanosis. Septic shock, multiorgan failure and disseminated intravascular coagulation ensue. 10-50% may develop hemorrhagic meningitis with headache, meningismus, delirium and coma, which can be the prominent feature upon presentation. Death usually occurs within 36 hours of the fulminant phase onset.

There are no specific laboratory tests to suggest anthrax, but hemoconcentration, manifested as an elevated hematocrit is common. The chest radiograph is always abnormal. The mediastinum is widened in at least 70% cases by the time of presentation, although it may be subtle at first. Pleural effusions and/or infiltrates probably corresponding to compressive atelectasis are found in greater than 80%. This is a chest x-ray from a naturally occurring case of inhalational anthrax showing the classic widened

mediastinum caused by lymphadenopathy, indicated by the shaggy borders, as well as a small left basilar infiltrate and effusion.

The differential diagnosis for inhalational anthrax is broad. Early disease mimics influenza and other upper respiratory virus infections, however nasal symptoms are typically not present and rapid diagnostic tests, such as nasal swabs for detection of respiratory virus antigens would be negative. In addition, the chest radiograph is always abnormal in inhalational anthrax, even during the early stages, whereas it would be expected to be normal in an uncomplicated case of viral upper respiratory infection. Pneumonia, especially from atypical pathogens, is high on the differential, however sputum production is minimal to absent in inhalational anthrax and there may be no pulmonary infiltrate. The widened mediastinum can be mistaken for primary mediastinitis or a dissecting thoracic aortic aneurysm, but lack of recent surgery and presence of fever should readily differentiate. The meningitis that occurs in many cases presents like other acute bacterial meningitides, however bloody CSF containing Gram positive bacilli should be seen.

Cutaneous disease usually occurs on the hands, arms, neck or head. The disease occurs whenever spores are able to penetrate the epidermis through visible breaks in the skin or microscopic cuts. The incubation period is typically 3-5 days, but may be up to 12 days, there is no clinical evidence of delayed spore germination in the skin. While mild constitutional symptoms can be seen, progression to severe systemic disease, preceded by lymphangitis and lymphadenopathy, is very rare when treated.

The hallmark of cutaneous disease is a single or few lesions that are painless throughout all stages, starting with a papule or macule that may be pruritic. Over the next 1-2 days a single or multiple vesicles or a large bulla appears with clear or serosanguineous fluid within. This dries into an ulcer with surrounding gelatinous, often extensive, nonpitting edema. If the edema involves the head or the neck, airway compromise can occur. Several days later this progresses to a black depressed eschar at the base of the ulcer. Purulence or significant erythema and evidence of inflammation should raise the suspicion of secondary bacterial infection such as cellulites.

This photo is an example of a typical cutaneous anthrax lesion revealing early black eschar formation with surrounding gelatinous edema. Cutaneous anthrax may be misidentified as a brown recluse spider bite and may be similar to ulceroglandular tularemia. There have been rare case reports of possible person-to-person spread of cutaneous disease.

Gastrointestinal disease is not well understood because there have been no reported cases in the United States. Gastrointestinal system involvement is not uncommon in the setting of systemic disease, but true GI anthrax is the result of primary germination within the GI tract. There are two recognized subtypes -- one is an oropharyngeal or esophageal type that occurs when inhaled particles larger than 5 microns land in the oropharynx and germinate, or ingested spores are deposited in the mouth or esophagus. This leads to regional lymphadenopathy and edema which cause stridor, sore throat, fever, dysphagia

and subsequent sepsis. The more common intestinal form follows ingestion of spores and subsequent germination within the small or large bowel. Early symptoms of nausea, vomiting and malaise, progress to hematochezia, bloody ascites and an acute abdomen. The differential diagnosis for gastrointestinal disease includes gastroenteritis, peritonitis, and any cause of acute abdomen.

Because of its mild, nonspecific nature, a high index of suspicion is necessary to make the diagnosis of anthrax in the early stages. This is compounded by the fact that there are no readily available rapid specific tests for confirming anthrax. The presentation of a previously healthy patient with severe flu-like symptoms and a widened mediastinum should prompt immediate treatment and diagnostic measures. The gold standard remains direct culture of clinical specimens onto blood agar with demonstration of typical colony, Gram stain, motility and biochemical features. A culture with the expected characteristics provides a preliminary diagnosis and greater than 90% confirmation that the organism is *Bacillus anthracis*. Direct culture of clinical specimens is widely available and should be performed on blood and other clinical specimens including pleural fluid and CSF, if available. Blood cultures should always be obtained prior to antibiotic initiation as they are positive nearly 100% of the time in inhalational anthrax, but rapid sterilization of blood after a single dose of antibiotics occurs. Sputum is unlikely to be helpful in diagnosing anthrax, but may identify a pathogen if the patient has a bacterial pneumonia instead of anthrax. If anthrax is suspected, the microbiology laboratory should be notified to ensure the workup of any Gram positive bacillus that is isolated. When growth does occur it is usually rapid, within 8-24 hours. Preliminary positive cultures are then sent to a reference laboratory for confirmation via gamma phage lysis and staining for *B. anthracis*-specific capsular or polysaccharide cell wall antigen. More rapid confirmatory tests available only at reference labs including PCR for all clinical specimens have the advantage of being able to confirm anthrax quickly and even when the cultures are negative. As the assays become more commercially available they may have an expanded role in first-line rapid diagnosis. Serologic testing can also be used to retrospectively confirm anthrax infection in cases that are culture-negative. Nasal swabs are a useful epidemiologic tool, but should never be used to rule out exposure to anthrax because of their low sensitivity.

The basic components of treatment for severe anthrax disease consist of hospitalization with intensive supportive care and IV antibiotics, which should be started immediately upon suspicion and prior to confirmation of the diagnosis. The 2001 outbreak confirmed that late diagnosis and treatment adversely affects prognosis. Steroids can be considered for treatment of all forms of anthrax when presentation is severe with significant edema, respiratory failure or meningitis.

As susceptibility data will be delayed, if available at all, antibiotics must be chosen empirically. Based on 2001 data and prior experiences, a recommended regimen for empiric therapy has been proposed, and should be followed until sensitivity patterns allow for adjustment. The CDC recommends the use of intravenous ciprofloxacin at 400mg q12h or doxycycline at 100mg IV q12h, in addition to one or two other antibiotics, choosing from clindamycin, vancomycin, rifampin, penicillin,

chloramphenicol or imipenem. Macrolides, cephalosporins and trimethoprim/sulfamethoxazole are usually ineffective and should not be used. The combination of ciprofloxacin, rifampin and clindamycin or vancomycin was successful in the 2001 outbreak. The same empiric therapy for inhalational anthrax should be provided to pregnant women as other adults, with the rationale being that the risks of the antibiotics on the welfare of the fetus are small and are outweighed by the benefits of treating suspected disease.

Empiric therapy for inhalational disease in children consists of the same drugs but dose adjusted for children at 10-15mg/kg/day of ciprofloxacin q12h with the maximum of 1g a day, and doxycycline at 2.2mg/kg which leads to an adult dosage for children greater than 8 years of age and greater than 45kg. One or two additional antibiotics should be added as in adults. When treating children, the risk to benefit ratio must be weighed, as the two primary drugs have real or theoretical toxicity in that population. The fluoroquinolones rarely cause arthropathy, and doxycycline carries the risk of dental enamel staining. However, in the setting where treatment is necessary for suspected or confirmed anthrax, the risk of the side effects are outweighed by the benefits of providing appropriate antibiotic therapy for this disease with such high morbidity and mortality.

Empiric therapy for cutaneous disease consists of the same drugs as inhalational and follows the same regimen if there are signs of systemic disease, extensive edema or if lesions are present on the head or the neck. Localized cutaneous infections can be treated with a single oral antibiotic, ciprofloxacin at 500mg bid or doxycycline at 100mg bid being the drugs of choice. Gastrointestinal disease should be treated similarly to inhalational.

For all forms of anthrax, the recommended duration of antibiotic therapy is 60 days because of the risk of delayed spore germination. Although delayed germination has not been seen with cutaneous disease, there may be a risk of coexisting inhalation of spores, depending on the mechanism of exposure. The clinical course should be monitored very closely for any evidence of recurrence after cessation of antibiotics. Intravenous antibiotics can be switched to oral antibiotics after clinical improvement is noted and the patient is able to tolerate oral medications. Upon switching to oral therapy the regimen should consist of one or two drugs, including the initially chosen ciprofloxacin or doxycycline, with guidance from susceptibilities. The treatment of children is an exception. Because of the uncertain benefit of treatment for a full 60 days, and the higher risk of drug toxicity from long-term use of fluoroquinolones and tetracyclines, amoxicillin can be considered for use upon switching to oral therapy. There is no role for anthrax vaccine in treatment of the disease.

Post exposure prophylaxis is the administration of antibiotics with or without vaccine after suspected exposure to anthrax has occurred but before symptoms are present. Once symptoms are present then it is considered active treatment. Post exposure prophylaxis should be offered to anyone who has had suspected direct exposure to aerosolized anthrax or powders containing anthrax. Prophylaxis should not be offered to contacts of cases unless they were also exposed to the original source. The decision to start post

exposure prophylaxis should be based completely on the risk of exposure and not on any laboratory tests, including nasal swabs, as none are sensitive enough to exclude the diagnosis or to reliably detect exposure. Antibiotics should be administered as soon as possible after exposure is suspected. Prompt therapy prior to symptom development is likely to decrease the risk of subsequent disease, and decrease the severity of disease that does develop. The antibiotics should be the same regimen as active treatment for cutaneous disease, with oral ciprofloxacin or doxycycline as the first line for empiric therapy. Ciprofloxacin is preferred over doxycycline in pregnant women because of potential adverse fetal effects, and because there is less certainty of the dramatic positive risk versus benefit ratio for prophylaxis as compared to treatment. If ciprofloxacin cannot be used for pregnant women, then amoxicillin should be the next choice. Because of the unknown magnitude of the risk for delayed spore germination (which was up to 98 days in primates), the known risk of adverse reactions with prolonged antibiotic therapy, and the possible benefit of administering vaccine, there are three options for duration of prophylaxis outlined by the CDC. These include 60 days followed by observation, 100 days and observation, or 100 days and vaccination.

Little data currently exists concerning the effectiveness of postexposure antimicrobial prophylaxis among exposed persons who have been partially or fully vaccinated. However, anthrax vaccine may be used in combination with antimicrobial PEP under an Investigational New Drug (IND) application with the Food and Drug Administration for unvaccinated persons at risk for inhalational anthrax. It is delivered in a 3-dose regimen (0, 2, 4) weeks. Use of anthrax vaccine for PEP could reduce the need for long-term antimicrobial therapy with its associated problems of nonadherence and adverse events. After the anthrax attacks in 2001, approximately 10,000 persons were recommended to receive a 60-day regimen of antimicrobial prophylaxis for suspected or confirmed exposure to anthrax, but adherence to the recommended 60-day antibiotic regimens was as low as 42%. Individuals who are partially or fully vaccinated may receive a reduced, more tolerable 30-day course of antimicrobial PEP.

The anthrax vaccine adsorbed (AVA) is a vaccine that has been in use in the United States for many years. Because of production problems, there is a limited supply that is controlled by the Department of Defense. It is an inactivated cell-free filtrate of the vaccine strain of *Bacillus anthracis*. The vaccine prevents disease in >95% of animals exposed to lethal aerosols. Although there are no human efficacy data, an older generation vaccine provided > 90% protection in cutaneous disease, and a trend toward protection versus inhalational. The vaccine does have some documented adverse effects, mostly as recorded from a database of over 1.6 million doses given to military personnel as of April, 2000. There were no deaths recorded and <10% resulted in moderate to severe local reactions including erythema and edema. Fewer than 1% had systemic reactions such as fever and malaise.

There is no known person-to-person transmission of inhalational anthrax, however there have been rare case reports of possible transmission of cutaneous disease. As the risk of transmission is thought to be extremely low, standard precautions are recommended for

patient contact, which includes the use of gloves when a draining lesion is present. Biosafety Level 2 (BSL-2) handling is all that is necessary for standard clinical specimens. However, for environmental samples, large volumes, or dealing with powders that could contain anthrax spores, BSL-3 handling should be used.