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Anthrax Bioterrorism: Prevention, Diagnosis and Management Strategies

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Abstract

Bacillus anthracis is one of a limited number of biological agents capable of causing death and disease in sufficient numbers to devastate an urban setting. In October 2001, reports of American patients with inhalational anthrax reacquainted the public with this ancient disease and introduced the harsh reality of a bioterrorist act. Cutaneous disease, gastrointestinal anthrax and inhalational disease are the known clinical manifestations of anthrax. Inhalational anthrax has an untreated mortality of nearly 100% and is the primary cause of death for an exposed and unprotected population. Vaccination affords a high degree of primary prevention and multiple effective antibiotics exist for both disease prevention and treatment. In this article, we will discuss the costs and effectiveness of prevention strategies, diagnosis, and therapy of bioterrorism related anthrax.

Key words: Bacillus anthracis; biodefense; Vaccine; Antibiotics; Cost-effectiveness

Introduction

The United States Working Group on Civilian Biodefense and the Centers for Diseases Control and Prevention (CDC) identify anthrax as one of a limited number of biological agents capable of causing death and disease in sufficient numbers to cripple a developed region or urban setting [1,2]. Research into the use of Bacillus anthracis as a bioweapon is at least 80 years old and several nations are believed to have weaponized anthrax [3]. The accidental release of aerosolized anthrax spores from a military microbiology facility in 1979 at Sverdlovsk, of the former Soviet Union, caused at least 68 deaths and demonstrated the lethal potential of aerosolized B. anthracis [4]. The World Health Organization and the United States Congressional Office of Technology Assessment have estimated that aircraft release of 50-100 kg of B. anthracis over a large city could result in 130,000-3 million deaths, many of whom would likely die before receiving treatment [5] [6]. More recently, the Aum Shinrikyo terrorist group is believed to have undertaken an ineffective release of aerosolized B. anthracis in Tokyo [7].

Twenty-two cases of bioterrorism-related anthrax were identified in the United States during September and October 2001 [8]. There were 5 fatalities involving 11 cases of inhalational anthrax and 11 cases of cutaneous anthrax [8]. Over 10,000 people potentially exposed to anthrax in Connecticut, Florida, New Jersey, New York City and Washington, D.C., were recommended to take post-exposure antibiotic prophylaxis [8,9]. The mortality from untreated inhalational anthrax approaches 100% and the costs associated with a real or perceived B. anthracis bioterrorist attack have been estimated at over $26 billion per 100,000 persons exposed [10-12].

Well before the 2001 attacks, the US Department of Defense had directed that all military services begin an anthrax vaccination program [13]. Live attenuated endospore-based vaccines were widely used in the former Soviet Union for both humans and livestock and remain in use in the Russian Federation [14]. In 1970, the US Food and Drug Administration licensed the anthrax vaccine adsorbed (BioPort Corporation, Lansing, MI) for human use [15]. The US military has now given more than 2 million anthrax vaccinations to more than 500,000 personnel since beginning the program, and an active system of side-effect and complication reporting has accompanied this program [16]. Although the US military has determined that its troops have sufficient risk of exposure to warrant the costs and side effects associated with vaccination, the optimal strategies for prophylaxis and treatment in a potential bioterrorist attack for civilian populations are highly controversial.

In this review, we will discuss the history of anthrax, its clinical manifestations, diagnosis and treatment strategies, and also evaluate the cost-effectiveness of anthrax prevention and treatment strategies for centers at risk for bioterrorist attacks.

Historical Background

Anthrax has long been considered a likely biological weapon [17-22]. B. anthracis may have been responsible for two of the plagues that affected Egypt in 1491 BC [23]. In 1877, Koch grew the anthrax bacillus in vitro and induced the disease in healthy animals by inoculating them with pure cultures of the bacillus [24]. John Bell recognized the link between handling infected wool and inhalational anthrax amongst textile workers. By setting standards for wool disinfection, he was influential in reducing the incidence of this disease in England [25,26]. The latter part of the 19th century also saw William Greenfield and Louis Pasteur develop the first anthrax vaccine for immunization of livestock [17,27]. The British conducted experiments by releasing spores of anthrax on Gruinard Island near Scotland in the 1940s. Viable anthrax spores persisted until the island was decontaminated with formaldehyde and seawater in 1986 [18]. The United States experimented with biological weapons including anthrax spores in the 1950s and 1960s until President Nixon's order to terminate the program was implemented in 1970 [20]. However, many countries continue to develop an offensive biological weapons program [28]. In the anthrax attacks of 2001, subsequently referred to as Amerithrax, B. anthracis spores sent through the US postal system resulted in 22 cases of anthrax [29]. The letter sent to Senator Daschle's office may have been responsible for two of the plagues that affected Egypt in 1491 BC [23].
have contained about 2 grams of powder and between 100 billion to 1 trillion spores of \textit{B. anthracis} per gram [30]. These Amerithrax attacks were the first known instance of delivery of anthrax spores through the postal system; a method of delivery less sophisticated than many of the scenarios tested by governments [21,30].

**Clinical Anthrax**

**Epidemiology**

\textit{B. anthracis} is found worldwide, mainly existing in the soil in the form of resistant spores. Anthrax is most prevalent among cattle, sheep, horses and goats. Infected animals may bleed from the nose, mouth and bowel, contaminating soil or water with \textit{B. anthracis} that can subsequently form spores and persist in the environment. Agricultural outbreaks are most likely to occur when the soil pH is not overly acidic and rich in organic matter and when conditions for multiplication are favorable. Changes in the soil microenvironment such as drought or rainfall enhance sporulation [18,31]. Animals contract the disease while grazing and the majority of naturally occurring human cases of anthrax are due to either agricultural or industrial exposure. There have been no reports indicating direct human-to-human transmission [30,32-37]. Workers in manufacturing plants using infected animal products, particularly contaminated hide, goat’s hair, wool or bone, are at highest risk [25,26,38]. Vaccination of animals has lowered the incidence of anthrax amongst livestock except in developing countries in Asia and Africa where vaccination programs are sporadic [17,39].

Three clinical forms of anthrax infection exist. Cutaneous anthrax is the most common, constituting over 95% of reported cases. An estimated 2000 cases of cutaneous anthrax occur worldwide annually and result from entry of spores through skin abrasions [30]. The largest reported epidemic of anthrax occurred in war torn Zimbabwe during 1979 – 1985, when the interruption of veterinary vaccination programs led to almost 10,000 cases of cutaneous anthrax [40]. Gastrointestinal anthrax disease is due to ingestion of contaminated undercooked or raw meat and is uncommon in the developed world [19]. While rare, inhalational anthrax accounts for the majority of morbidity and mortality associated with \textit{B. anthracis} infection. In 1976, the last naturally occurring case of inhalational anthrax reported in the United States, resulted in the death of a craftsman working with infected yarn imported from Pakistan [31]. The rarity of this disease in developed countries makes even a single new case of inhalational anthrax, especially one without any identified natural exposure, alarmingly suspicious.

**Microbiology**

\textit{B. anthracis} is the bacteria responsible for anthrax. It is a large (1 to 1.5 \textmu m wide and 4 to 10 \textmu m long), square ended, nonmotile, aerobic, Gram-positive rod, with a centrally located spore. Spores are approximately 1 \textmu m in size. The spores appear as unstructured areas on Gram stain preparations. In vitro, the bacteria frequently occur in long chains giving them a jointed bamboo rod appearance. In the host, \textit{B. anthracis} appears as individual organisms or chains of 2 to 3 bacilli. Microscopically, the chains are usually surrounded by a capsule. An India ink stain may better visualize the capsule [18,30,41,42]. Plated colonies of \textit{B. anthracis} are usually large (4-5mm), opaque and irregular with characteristic comet tail protrusions [18,42]. \textit{B. anthracis} can be isolated from blood, sputum, cerebrospinal fluid (CSF), vesicular fluid and stool.

\textit{B. anthracis} grows on blood agar plates, usually within 24 hours. Optimal growth temperature for the organism is 35°C (range 12-45°C), in a pH range of 7.0 to 7.4. When grown above 45°C the bacteria become attenuated and avirulent due to loss of the capsule [18]. Several biochemical tests aid in differentiating \textit{B. anthracis} from other members of the species. \textit{B. anthracis} is characterized by the absence of hemolysis on sheep blood agar, lack of motility, absence of salcin fermentation, gelatin hydrolysis, and lack of growth on phenyl ethyl alcohol medium [42,43]. Hospital laboratory diagnosis is presumptive based on Gram stain characteristics and the growth of large non-motile, non-hemolytic colonies on sheep’s blood agar cultures. Level B laboratories belonging to the Laboratory Response Network for Bioterrorism perform additional tests to confirm the presence of \textit{B. anthracis} including lysis by gamma phage, direct fluorescent antibody staining of the capsule and detection of the cell-wall polysaccharide antigen [32,42].

Sporulation requires oxygen, but germination of spores does not. Spores form only on culture plates, soil and tissue of dead animals after the nutrient supply is exhausted. Spores germinate in an environment rich in amino acids and glucose such as in the blood or tissues of infected living animals or humans. Spores are highly resistant to drying, boiling for 10 minutes, and most disinfectants. Heating at 120°C for at least 15 minutes is used to inactivate the spores, yet they often survive for many years in arid and semi-arid environments [43].

**Pathogenesis**

Inhalational anthrax results when spores of \textit{B. anthracis} enter the human body by deposition into alveolar spaces; cutaneous anthrax occurs when spores settle into abrasions or cuts on the skin; and gastrointestinal anthrax develops after ingestion of raw infected meat containing a large number of vegetative bacteria. In each location, macrophages then ingest spores and spores then release toxin which ultimately leads to cell death [44].

The capsule contains the principal virulence factors of \textit{B. anthracis}, specifically, poly-D-glutamic acid, and anthrax toxin. The capsule plays a key role in providing resistance to phagocytosis [17,44]. Anthrax toxin consists of three proteins called protective antigen (PA), edema factor (EF), and lethal factor (LF) [45]. These virulence factors are found on two large plasmids, pXO1 and pXO2. pXO1, 184 Kb in size, contains the genes that produce anthrax toxin complex and their transcriptional regulators. pXO2 is 97 Kb in size and contains the genes responsible for capsule synthesis [44]. Protective antigen is named for its ability to provide experimental protective immunity against \textit{B. anthracis}. It is an 83-Kd protein that binds to target cell receptors. A small 20-kd N-terminal fragment is proteolytically cleaved allowing the larger cell bound PA fragment to act as a membrane channel. EF and LF bind to exposed sites on the PA fragment and form edema toxin and lethal toxin. PA then transfers these enzymatic proteins across cell membranes and releases them into the cell cytoplasm where they exert their effects [18,31,44,45].

Edema factor is a calmodulin-dependent adenyl cyclase that converts adenosine triphosphate to cyclic adenosine monophosphate. Edema toxin plays a role in inhibiting both phagocytic and oxidative burst activities of polymorphonuclear leukocytes and interferes with water homeostasis leading to edema [46]. Lethal factor and the subsequently formed lethal toxin stimulate macrophages to release tumor necrosis factor α and interleukin-1β (IL-1) [47]. At high concentrations lethal factor causes lysis of macrophages [17,44,47]. Pro-inflammatory mediators are formed and stored within the macrophage early in the course of anthrax infection, when toxin levels are lower than the critical concentration required for lysis. Later as the infection progresses and the number of bacteria increases, a threshold for lysis is reached and large amounts of preformed mediators such as IL-1, IL-6,
TNF-alpha are released in the circulation, possibly accounting for the sudden death seen in anthrax victims [30,46].

Scientists have not determined the molecular target of LF and EF [48]. Several animal experiments identify the important role of macrophages as cellular mediators. In one study, silica injections were used to deplete macrophages in mice. Silica treated animals became resistant to lethal toxin and there was 100% survival in the silica treated group compared to less than 10% in the control group. The mice became sensitive to lethal toxin again after cultured macrophages were re-introduced into the experimental group; infusion of other cell lines did not have any effect [44,49].

**Clinical Presentation**

The entry of *B. anthracis* spores into exposed skin through cuts and abrasions results in cutaneous anthrax. Spores germinate in the tissue and vegetative cells quickly multiply and produce anthrax toxin resulting in local edema. Small, painless, often puritic papules form initially and by the second day enlarge to form 1-3 mm vesicles. Clear or serosanguineous fluid is discharged from the vesicles. Gram stains of the fluid may show a few leukocytes and many Gram positive rods. Painful regional adenopathy, fever, and malaise may occur. The lesion ulcerates and progresses to a characteristic painless black eschar that lends this disease its name - anthrax is derived from the Greek word for coal, the color and appearance of the eschar. The eschar heals in 1-3 weeks. Antibiotics do not seem to increase the rate of healing but they decrease the instances of systemic disease [17,24,30,50]. Death from cutaneous anthrax is rare if treated promptly; however, without antibiotics mortality approaches 20% [32]. In the Amerithrax experience, 11 suspected or confirmed cases of cutaneous anthrax occurred with painless, pruritic lesions occurring on the neck, chest, forearm and fingers. The incubation period, ranged from 1 to 10 days. None of the cases of cutaneous anthrax diagnosed in 2001 were fatal [51].

Gastrointestinal anthrax symptoms appear 2-5 days after ingestion of contaminated undercooked meat. Bacteria are transported from the bowels to mesenteric and regional lymph nodes. Oral and esophageal ulcers can occur. Regional lymphadenopathy and edema may rarely lead to airway compromise [52-54]. Lower intestinal disease includes lesions in the cecum and terminal ileum, hemorrhagic adenitis and occasionally massive ascites[52-56]. Nausea, vomiting, abdominal pain, hematemesis, hematochezia and sepsis are all presenting features. Early diagnosis is difficult and the mortality is high [17,57,58]. Lack of clinical experience with this rare but lethal form of anthrax leads to airway compromise [52-54]. Lower intestinal disease includes lesions in the cecum and terminal ileum, hemorrhagic adenitis and occasionally massive ascites. Early diagnosis is difficult and the mortality is high [17,57,58].

The majority of recent experience with inhalational anthrax derives from the Amerithrax attacks and the 1979 Sverdlovsk exposure [56,59]. Aerosolized *B. anthracis* spores that are between 2 and 5 μm in size reach the alveolar ducts and alveoli. Pulmonary macrophages ingest and transport the spores to mediastinal and hilar lymph nodes where germination and toxin production ensues. The toxin is released into the systemic circulation, resulting in edema, hemorrhage, necrosis, septic shock and death [17,32]. Spore germination in the mediastinum results in hemorrhagic mediastinitis, a hallmark of inhalational anthrax. The minimum dose of anthrax spores sufficient to cause infection in humans is unclear. One study found that the minimum infectious inhaled dose in chimpanzees is 40,000 to 65,000 spores [60]. Estimates for humans suggest that between 2500 -10,000 inhaled spores are sufficient to kill 50% of exposed persons [20,30]. The specific amount of anthrax spores involved in the 11 cases in Amerithrax is not reported, however, circumstances surrounding the exposure suggest that for some patients the infectious dose may have been lower than the previously estimated 2500 spores [30]. Patients with a previous history of cardiopulmonary disease are at increased risk for inhalational anthrax [39].

There are two distinct clinical stages of inhalational anthrax. The initial phase follows an incubation period of 1 to 6 days and begins with the insidious onset of non-specific symptoms such as myalgia, malaise, fatigue, non-productive cough, intermittent retrosternal pressure, and low-grade fever. This stage continues for 2-4 days and the patient may even appear to improve after the first few days [17,18,30].

The following stage has a quick onset and death may occur within 24 to 36 hours. Patients become acutely short of breath and rapidly develop shock, hypoxemia and cyanosis. Mediastinal lymph node enlargement may lead to partial tracheal compression and airway compromise. Auscultation of the lungs is remarkable for crackles and signs of pleural effusions [18,25,26,30,32,61]. Up to half of the patients may develop hemorrhagic meningitis with meningismus, decreased level of consciousness and coma [17,18,30]. The clinical presentation of 10 of the 11 inhalational Amerithrax cases is consistent with previous reports in the literature [30,41,62,63,64,65]. The chest radiograph [212] and computed tomography (CT) [213] of the thorax [30,66] are useful in developing clinical suspicion of anthrax [66,67,68]. CT of the thorax should be particularly considered when the chest radiograph is normal but the clinical suspicion for inhalational anthrax is high [41,62,63,69,70].

**Diagnosis and Treatment**

**Diagnosis:** As the initial symptoms of inhalational anthrax are non-specific, diagnosis can be difficult; the early stages of the disease may resemble the common cold or other viral infections. An ideal detection system should be very sensitive (detects a low number of organisms or spores), specific (has low no cross-reactivity with other organisms or similar antigens), can be done rapidly on available specimens, simple to perform (not requiring extensive equipment or highly trained personnel), and cost-effective [71]. Although a number of available techniques fulfill some of these criteria, none satisfy all. Most assays are based on detecting the entire organism, organism antigens, or organism nucleic acid through one of the following techniques: culture-based conventional methods; immunological detection; nucleic-acid based assays; ligand-based detection; and biosensors.

Conventional culture methods, as described, are limited by imperfect sensitivity (for most patient samples), a labor-intensive process and a delay in diagnosis. Immunological enzyme linked immune assays are based on the concept that any compound able to trigger an immune response can be targeted as an antigen [71] and are increasingly used for detection of spores, vegetative cells and toxin proteins. If such assays are based upon antibody detection, they are relevant only in later stages of the infection, as opposed to early diagnosis. As well, many still have limited sensitivity thresholds of 100-1000-fold above infectious doses. There are numerous polymerase chain reaction (PCR) amplification of organism nucleic acids techniques available. PCR is more sensitive than most antigen-antibody-based detection systems, and can be used in real-time. PCR is very specific, depending upon the nucleic acid probes selected; however, can be affected by high concentrations of competing antigens, nucleic acid and salts and biological substances from other sources, and is therefore best performed on cultured organisms as opposed to many bodily fluids. A variety of promising biosensors also exist for the environmental detection of organisms.
or spores and are commonly based upon nucleic acid probes and antibody sensors in combination with evanescent wave fiber-optics, electrochemiluminescence, quartz crystal microbalance and cantilevers

How then should clinical diagnosis be approached for patients suspected of infection? Initial evaluation should include white blood cell count, chest imaging, and despite the limitations of conventional culture methods, they should still be done due to the wide spread availability and experience with interpretation of Gram stains and cultures of appropriate specimens [69]. Yet, as anthrax does not cause a classic bronchopneumonia, sputum from patients seldom yields positive smears or cultures. Only 1 patient in the Amerithrax attacks had a positive sputum Gram stain [30]. Nevertheless, suspicious Gram stains should be reported to expert laboratories for further evaluation [69]. Clinicians should alert the hospital laboratory of the clinical suspicion of anthrax, allowing for simple biochemical testing and morphological evaluation of the colonies and permitting a presumptive diagnosis within 12-24 hours of inoculation of cultures. Serological diagnosis of anthrax can be made by means of a microhemagglutination test specific for the protective antigen component of the toxin [30,32]. Rapid screening immunoassays (most commonly ELISAs) and PCR based techniques as described above should be used in laboratories with access to such techniques.

Antibiotic Therapies

Early antibiotic therapy reduces the mortality. Recommendations for the use of antibiotics are based on limited human experience and experimental studies on animals (Table). Naturally occurring B. anthracis strains are sensitive to penicillin but resistant to extended spectrum Beta-lactam antibiotics (third generation and fourth generation cephalosporins), sulfamethoxazole, trimethoprim, and aztreonam [30]. The US FDA has approved penicillin, doxycycline and ciprofloxacin for the treatment of inhalational anthrax [18,30,32]. Other fluoroquinolone antibiotics likely have similar efficacy as ciprofloxacin but animal studies are lacking [30]. Following the Amerithrax attacks, CDC recommended the use of 2 or 3 antibiotics in combination based on limited information that patients on combination therapy had a better chance of survival [63]. While the isolates obtained from patients with inhalational anthrax in 2001 were susceptible to all expected antibiotics, future genetically engineered B. anthracis strains may be resistant to 1 or more antibiotics. Combination therapy would be prudent in such instances. Some infectious disease specialist recommended the addition of clindamycin to the treatment regimen. Despite the lack of data for its use in anthrax infection, clindamycin may confer the theoretical advantage of reducing bacterial toxin production [30]. In the case of suspected or proven meningitis some experts recommend the use of ciprofloxacin over other antibiotics due to its better central nervous system penetration. Augmentation with rifampin, chloramphenicol or penicillin may also be beneficial [30]. Intravenous antibiotics at doses listed are preferred over oral antibiotics (Table). Oral antibiotics may be used once the patient is clinically stable. In cases of a mass bioterrorist attack and when intravenous supplies are exhausted Ciprofloxacin 500 mg orally every 12 hours, or Doxycycline 100 mg orally every 12 hours is recommended as part of combination therapy [30]. Therapy should be continued for 60 days. Fluoroquinolones are generally not recommended for pregnant women or children due to the risk of arthropathy. No controlled studies of ciprofloxacin use in pregnant women are published. Balancing the life threatening risks of inhalational anthrax and potential adverse drug effects, the working group on civilian biodefense [30] and the CDC, [72] recommend the initial use of ciprofloxacin as a component of combination therapy in pregnant women and children. Amoxicillin may be used for treatment only after 14-21 days of fluoroquinolone or doxycycline administration. If doxycycline is used in pregnant women, periodic liver function tests are recommended [30]. Despite some evidence that doxycycline has resulted in discolored teeth and skeletal growth retardation in infants, the consensus remains that this drug may be used in this population if the use of ciprofloxacin is not possible [30].

Intensive care units, where careful attention is paid to the management of hypotension and providing adequate ventilatory support, are essential in the successful care of patients with inhalational anthrax. Large pleural effusions may worsen respiratory distress and need repeated drainage or chest tube placement [30]. There is no data to suggest person-to-person transmission of the disease [17,30,59]; thus standard isolation precautions are recommended for all patients with anthrax and there is no need for airborne precautions using special masks. Household contacts of patients with anthrax do not need to receive prophylaxis unless they too have a known or suspected exposure [30].

Recombinant anti-PA Antibodies

Raxibacumab is a human IgG1 monoclonal antibody directed against B. anthracis PA. It binds PA with high affinity and specifically blocks the binding of PA to its receptor, preventing anthrax toxin-mediated damage. Studies in rabbits and monkeys, have shown that raxibacumab improves survival in animals with evidence of systemic disease after an inhalation of a lethal dose of anthrax spores [73]. While these studies have not been repeated in humans the same investigators have shown that an intravenous dose of 40 mg/Kg of raxibacumab in humans results in levels of serum raxibacumab that are similar to or greater than those that provide a survival benefit in animal models. Moreover, the safety profile in humans is acceptable with only one serious adverse event (cholecystitis) observed in 333
subjects, thought possibly related to treatment. Other adverse events were mild to moderate in severity and transient; their incidence did not differ significantly between treatment and placebo groups. Although antibiotics remain the mainstay of initial treatment after exposure to \textit{B. anthracis}, early intervention (before significant increase in PA levels) is associated with better survival in animals. In patients with a high clinical index of suspicion for inhalational anthrax infection, or for those with poor clinical response to antibiotics, the use of raxibacumab concomitantly with antibiotics may be prudent.

\section*{Vaccination and post-exposure prophylaxis}

The CDC issued guidelines during the Amerithrax attacks for the management of individuals with suspected or confirmed aerosol exposure to \textit{B. anthracis} [34]. The antibiotics recommended for prophylaxis are the same as those used for treatment and are prescribed for 60 days (Table). No evidence exists to suggest superiority of ciprofloxacin over doxycycline for anthrax prevention. Widespread use of either antibiotic may lead to resistant strains and patients who need prophylaxis should be chosen based on available and evolving laboratory and epidemiological evidence [30,34]. Antimicrobial prophylaxis is not currently recommended for hospital personnel taking care of patients with inhalation anthrax, or for pathologists performing autopsies on bodies with anthrax when appropriate isolation procedures are followed [34].

The US anthrax vaccine is manufactured by the Bioport Corp (Lansing, Michigan) and is produced from cell-free filtrates of bacilli. The PA content of the vaccine is high and is mainly responsible for inducing immunity [27,74]. The vaccine is administered subcutaneously at 0, 2 and 4 weeks, and 6, 12, and 18 months, followed by yearly boosters and data regarding the safety of the vaccine is readily available [74,75] and generally favourable. At least 1,859,000 doses of anthrax vaccine were used in the United States from January 1, 1990 to August 31, 2000, and there were 1,544 reports of adverse events [74]. The most frequently reported events were hypersensitivity, edema, and pain at the injection site, headache, arthralgia, and pruritis. Analysis of the data did not find a pattern of serious adverse events requiring hospitalization. Adverse events were self-reported and there is insufficient information in peer-reviewed literature to determine whether anthrax vaccine has any long-term adverse health outcomes, although no data to-date would indicate this to be likely [74].

We must rely heavily upon animal studies when considering the effectiveness of pre-exposure anthrax vaccination. In one study, rhesus monkeys received two doses of vaccine prior to exposure to a lethal dose of aerosolized anthrax spores. All monkeys in the non-vaccinated group died within 5 days of exposure, while the vaccinated monkeys were protected up to 2 years [17]. A controlled, single blinded clinical trial in goat hair mill workers, at risk for cutaneous anthrax, evaluated vaccine efficacy in humans [76]. During the study there was an outbreak of inhalational anthrax, no cases occurred in participants who were vaccinated; 5 cases of inhalational anthrax were documented among persons who were either receiving placebo or were not participants. The overall efficacy of the vaccine (a precursor of the currently available formulation) was 92.5\% for the combined endpoint of cutaneous and inhalational anthrax [75,76]. The FDA continues to “view the vaccine as safe and effective for persons at risk of exposure” and there is no data suggesting adverse additional adverse effects or risk in pregnant patients [75].

The US anthrax vaccine is currently not licensed for use as post-exposure prophylaxis, although it was used in the Amerithrax attacks under investigational new drug procedures [30]. Animal data suggests that this vaccine has a role, in combination with antibiotics, in preventing anthrax infection once exposure has occurred. Six groups of monkeys were exposed to a lethal dose of aerosolized anthrax. After the first day of exposure, groups were either given vaccine alone, saline placebo, antibiotics alone or a combination of doxycycline and vaccine. Survivors were re-challenged with aerosolized anthrax 131-142 days after initial exposure. On re-exposure, the majority of the monkeys that received vaccine alone died; none of the monkeys on combination therapy (vaccine and doxycycline) died [77]. On the basis of this study, the US working group for civilian biodefense recommends that “vaccination of exposed persons following a biological attack in conjunction with antibiotic administration for 60 days following exposure provide optimal protection to those exposed” [30]. During the Amerithrax attacks, vaccination was not offered to all persons suspected to be exposed; the shortage of sufficient quantities of vaccine may have been a reason for this omission. Vaccine was offered to those individuals considered at high risk for exposure towards the end of their 60-day antibiotics course. These individuals were also given the option to extend their antibiotic therapy without vaccination or have regular medical follow up without any further therapy [30]. No new cases of inhalational anthrax were reported in these individuals.

Cost-effectiveness of preventative therapies for Anthrax in the setting of bioterrorism attacks

In order to evaluate the costs and effects of various preventative strategies in the setting of Anthrax bioterrorist attacks, authors have developed mathematical models incorporating variables important in the probabilities of attack, exposure and resultant disease, associated quality of life and life-expectancy of individuals at risk, as well as near term and long term health and non-health related costs [78]. These models allow for simulation of events that have not occurred, exploration of the important drivers of model outcomes, and permit sensitivity analysis through modification of key variables. Results help to inform individuals, health care personnel and policy makers on optimal treatment strategies if such events do occur.

We developed a decision analytic model to compare outcomes of pre-attack and post-attack anthrax prevention and treatment strategies for urban centers at risk of a large-scale bioterrorist attack, of greater potential magnitude than experienced through the United States postal service in 2001 [78]. This analysis followed the recommendations of the Panel on Cost effectiveness in Health and Medicine, and adopted a societal perspective for costs and benefits, discounted at 3\% annually [79]. A decision model captured the costs and benefits of the different strategies immediately following an attack and for the remaining expected lifespan of an individual within the cohort. The costs, harms and benefits of two pre-attack strategies: vaccination with the human anthrax vaccine versus no vaccination; and four post-attack strategies: no vaccination, vaccination alone, antibiotic prophylaxis alone, or vaccination and antibiotic prophylaxis were compared. This model followed a hypothetical cohort of persons residing or working in a large metropolitan US city with a gender distribution (53\% female), mean age (36 years), and age-specific life-expectancy similar to that of New York City [80,81]. Base case probabilities were determined following an extensive review of the literature and after consultation with experts in public health and bioterrorism preparedness planning. The authors assumed a 1\% per year baseline probability of attack and 10\% population exposure to \textit{B. anthracis} spores if an attack occurred. Such an attack rate is arbitrary and unknowable; however were based upon a survey of experts who believed that for the nature of attack considered
(“a large-scale aerosolized release of B. anthracis over a U.S. metropolitan area”) a higher rate (e.g. 5-10% per year) was too high yet there was and continues to be a real and non-zero risk. Because exposure rates will likely vary substantially based on type of attack and local environmental factors, these estimates were varied widely in sensitivity analyses.

The probability of surviving clinical anthrax was estimated from reports of the recent US anthrax cases [65,82] and clinical outcomes of similar disease states [83,84,85]. Without prophylaxis, clinical disease given a sufficient spore inhalation is nearly uniform, and the mortality from inhalational anthrax approaches 100%; therefore 95% of individuals who had sufficient exposure during an attack would develop severe inhalational anthrax without prophylaxis or vaccination [1,8,11,22,37]. With post-attack treatment, the case-fatality rate of the inhalational anthrax contracted through letter contamination in 2001 was 45% [1,37,65,86]. Pre- and post-attack antibiotic and vaccination strategies further attenuate this risk [87]. Appropriate prophylactic antibiotics may prevent disease in greater than 80% of patients, depending upon individual adherence, thus 20% of people may still develop disease in this scenario [1,8,37,86]. Age-specific life expectancy in the absence of a bioterrorist attack was derived from US Life Tables [88].

Varying antibiotic choices and costs were considered within the model and through sensitivity analyses. Rapid distribution and dispensing of post-attack therapy, previously shown essential in limiting morbidity and mortality after an anthrax bioterrorist attack, was assumed [87]. However, recognizing that there may be logistical distribution problems in the aftermath of a bioterrorist attack, the effects of delay in distribution and dispensing were also explored. The US Advisory Committee on Immunization Practices endorses making the anthrax vaccine available in a 3-dose (0, 2 and 4 week) regimen, in combination with antibiotic therapy for post-exposure prophylaxis [89]. However, immediately following a bioterrorist attack, immunity would initially be incomplete and it was estimated that post-attack vaccination would be half as effective as immunity provided by the full course of vaccination; corresponding to the observed survival reduction in animal models given a single inoculation, prior to aerosolized exposure of B. anthracis [90].

Medical cost estimates in the model included costs associated with prophylactic vaccination and antibiotic therapy, in-patient and outpatient medical care, potential lost earnings, death costs and age-specific medical costs; all adjusted to 2004 US dollars using the gross domestic product deflator [91]. The cost of the vaccine is not well known, but previously has approximated $18 for a complete immunization series ($3 per dose) [92]. The cost of administering the vaccine series is higher if the vaccine is given by individual clinicians than as part of mass public vaccinations. For the base-case analysis, estimates from mass vaccination programs were used [93-96]. Expenditures for adverse reactions based on estimated out-patient and in-patient costs, medication costs, and loss of work costs associated with commonly reported adverse reactions were included [12,97,98,99]. Inpatient medical costs were derived from a review of the published records of hospital stays and care for patients with cutaneous and inhalational anthrax due to Amerithrax [65]; and, from comparison of the costs of care for similar disease states, derived from the Centers for Medicare and Medicaid Services Provider Specific File [8,12,85,98,100,101,102]. Future health care costs for patients who survived the initial anthrax illness were estimated using adjusted age specific medical expenditure data from the 1998 Statistical Abstract of the United States [88,103].

Adjusted life expectancy for quality of life using health state utilities was employed for all models [104,105] adapting utilities for clinical anthrax from similar disease states [106].

Pre-Attack Vaccination versus No Vaccination Strategies: For a scenario in which the annual risk of attack was 1%, and 10% of the population had an exposure sufficient to cause infection, the no vaccination strategy was less expensive and resulted in marginally higher quality of life-years gained (QALYs) per person. The probability that any individual received a clinically significant exposure in this scenario was the product of the probability of an attack (1%) and the probability of exposure given an attack (10%) or 1 in 1000. At this risk of attack, the pre-attack vaccination strategy cost an additional $163 per person, but reduced QALYs by 0.01. The adverse effects of the vaccine outweighed the benefits associated with its ability to provide protection against anthrax. For a city with a population of 5 million the incremental cost of prior vaccination would be $851,000,000 without appreciable health benefits. Although pre-attack vaccination of urban centers was not an effective strategy if the combined probability of exposure was 1 in 1000, prior vaccination did provide net health benefit when probability of exposure for an individual was greater than 1 in 500. However, the incremental cost effectiveness ratio of pre-attack vaccination of the population fell to $50,185/QALY only when individuals had a combined probability greater than 1 in 200 (Figure). Although there is no “threshold”, above which therapies should be considered cost-effective or not, interventions associated with an incremental cost-effectiveness ratio near or under $50,000/QALY are often considered reasonable value for the health care dollar [78,79].

Post-Attack Vaccination and Antibiotic Prophylaxis Strategies: Four post-attack strategies have been considered: no prophylaxis, antibiotic prophylaxis alone, vaccination alone, and both antibiotics and vaccination. No prophylaxis was the least effective and most expensive strategy. Costs were higher in the absence of vaccination or antibiotic prophylaxis because of the high cost of treating inhalational anthrax. Vaccination alone was less effective than antibiotic prophylaxis alone. Use of both antibiotic prophylaxis and vaccination was the most effective strategy, and had lower costs than other strategies. The combination strategy was less expensive because it prevented more cases of inhalational anthrax and prevented more deaths than did either of
the individual strategies. Although vaccination was not a cost effective pre-attack strategy at baseline risk of exposure, if individuals or policy makers did choose or recommend prophylactic vaccination, then the least expensive and most effective post-attack strategy would similarly be to add antibiotics, with a lifetime cost-savings of $335 and 0.33 additional life-years gained, per person treated with both vaccination and antibiotics, as compared to pre-attack vaccination alone. The efficacy of vaccination after a bioterrorist attack may vary depending on the timing of vaccination and the strain of anthrax used. However, even when vaccination added only marginally increased effectiveness to antibiotic therapy (e.g. vaccine efficacy < 10%), a combination of vaccination and antibiotic therapy remained the most effective and least expensive strategy. The additional effects gained through vaccination when an attack has occurred (even at low vaccine efficacy), outweighed any effects lost through vaccine-associated side effects.

The authors also assessed the sensitivity of the model to estimates of the cost of antibiotic and vaccination therapies [78]. Assuming similar efficacy among recommended antimicrobials, the least expensive medication (doxycycline) was always the dominant treatment strategy. However, when varying the drug cost between the least and most expensive antibiotic considered (ciprofloxacin), the incremental cost effectiveness of strategies containing antibiotic prophylaxis remained under $20,000/QALY, compared to a strategy of vaccination alone. Even if the cost of the vaccine increased to $150 per dose, the strategy of vaccination plus antibiotic prophylaxis remained the most effective strategy and cost $7000 per QALY gained as compared to antibiotics alone, if an attack did occur.

As adherence to antibiotic therapy fell from a base case of 100%, to below 50%, the strategy for forgo prior vaccination was no longer dominant. However, adherence to the suggested regimen would need to fall to approximately 20% before the incremental cost effectiveness ratio of prior vaccination approached $100,000/QALY gained, depending upon the specific nature of non-compliance. Similarly, if the ability to distribute antibiotics for prophylaxis after a B. anthracis bioterror attack is limited, and fewer than 50% of the exposed population were able to receive antibiotics (i.e. if antibiotic prophylaxis could be delivered to fewer than 250,000 of the 500,000 people exposed in a city of 5 million people), then prior vaccination became cost effective at a $100,000/QALY gained threshold, underscoring the importance of an organized and timely treatment distribution plan.

Cost-effectiveness limitations and considerations: Successful response to an aerosolized B. anthracis bioterror attack requires a strategy for recognizing that an attack has occurred and for timely delivery of vaccination and antibiotic therapy to a large population. Results suggest that if distribution of antibiotics or vaccinations compliance is substantially impaired, prior vaccination may become cost effective. Additionally, antibiotic prophylaxis would still be a cost effective component of post-attack strategy, even for those who had previously received vaccination. These findings highlight the critical need for distribution systems that can provide prophylaxis and vaccination rapidly for hundreds of thousands, perhaps millions of exposed people. They also highlight the inherent difficulties in decision making about anthrax vaccination. For a large metropolitan US city, vaccination provides net benefit when the probability of significant exposure (which is the product of the probability of an attack, and the probability of exposure given an attack) reaches about 1 in 200. Although it is difficult to judge the likelihood of a release and the probability of exposure given a release, clearly some individuals are at higher risk than others. The finding that at even relatively low probabilities (1 in 500), vaccination provides net health benefit may help decision makers assess the desirability of vaccination of military and emergency services personnel likely at greater risk of exposure than the general population. If a vaccine with fewer adverse reactions became available, the probabilities of exposure at which there may be net benefit would be lower.

Importantly, because the use of Raxibacumab in humans has very promising but limited efficacy and follow-up data, we did not include it in models reported here. Separate studies of the costs and effects of Raxibacumab, and considerations about optimal use among patients with possible or confirmed inhalational anthrax represent important next steps.

Expert opinion

Planning for bioterrorism presents a huge challenge to health care systems, industry, government and the military. There is tremendous expense in planning for all disparate possibilities and governments are frequently forced to focus energy and capital on those threats that are most likely, most devastating and frequently, those highlighted by recent experiences. Ironically, while the mere act of planning for a specific bioterrorist possibility may make the threat less likely, it may provoke modification of an attack to minimize the effectiveness of such planning.

There are many important considerations in the event of an anthrax attack. With respect to prevention, we will certainly require a stable production and supply of vaccine, ideally with ability to boost production in times of increased demand. This vaccine should undergo further animal testing and field trials of effectiveness in individuals at higher than average risk of exposure. Similarly, nations require the ability to stockpile, and to replete a stockpile, of antimicrobials most useful for anthrax. Medications such as ciprofloxacin and doxycycline are appealing from the perspective of anthrax (B. anthracis); and doxycycline is especially appealing given its activity against other threats such as plague (Y. pestis), brucellosis (Brucella spp.), Q fever (C. burnetii), and Tuleremia (F. tularensis). Ciprofloxacin has added advantage as a broad-spectrum antibiotic, with very good bioavailability and activity against the many Gram negative organisms that cause disease in humans, particularly Gram negative enteric organisms. Doxycycline is equivalent or better in activity against atypical organisms causing outbreaks of community acquired pneumonia.

In responding to any bioterrorist act, rapid detection of the agent will be important to limit early morbidity and mortality and to allow for a focused prevention and therapeutic response. Field or laboratory rapid diagnostic systems should be a focus of research and development. Similarly, regardless of the threat, government agencies and response teams will require rapid and effective means of mobilization of medication (both antibiotics and vaccine), and personnel to affected areas. The medication, personnel and protective equipment will vary depending upon the known or perceived threat, but most likely be employed rapidly in order to contain and treat the specific threat. An adjunct to organized governmental and agency response might include bioterrorism self care kits including vaccine and easy means of inoculation in addition to a supply of antimicrobial agents. Clearly, if a specific event disrupts the ability of a population or system to function overall (food, water, sanitation, health care), provisions must be made deal with this (rations, water purification systems, evacuation, etc.)

Conclusion

Anthrax, a disease of antiquity, has made headlines in the 21st
century through an intentional act of bioterrorism. *B. anthracis*, the bacterium responsible for this disease, is a rod shaped, spore forming, non motile bacillus. Inhalational anthrax is the clinical entity that leads to the highest morbidity and mortality. Early diagnosis is difficult and a high index of suspicion is required – cultures of blood, sputum or other bodily fluids provide a presumptive diagnosis in most patients. Combination therapy with appropriate antibiotics and intensive medical care decrease mortality. The US FDA has approved penicillin, doxycycline and ciprofloxacin for the treatment of inhalational anthrax. *B. anthracis* is naturally resistant to third generation cephalosporins, sulfamethoxazole, trimethoprim, and aztreonam. Unfortunately, mortality of this disease remains high. In limited trials and more extensive military use, the commercially available vaccine appears effective and generally safe. Under low baseline probabilities of bioterrorist attack and exposure, mass prophylactic vaccination is unlikely to be cost-effective. For individuals or groups with a greater than 1 in 200 risk of exposure, vaccination may become cost-effective. If there is inadequate or impaired delivery of prophylactic antimicrobial therapy to those potentially exposed, or if adherence to suggested regimens is low, mass vaccination may become cost-effective. Although antibiotics remain the mainstay of initial treatment after inhalational exposure to *B. anthracis*, Rabxidacumb, a human IgG1 monoclonal antibody directed against *B. anthracis* PA is a promising new therapy.

References


