Pulmonary Manifestations of Bioterrorism

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Along with smallpox, inhalation anthrax and pneumonic plague are among the diseases most likely to be spread by biowarfare, either from a rogue nation or terrorist group. Neither anthrax nor plague has been seen by many pulmonary (or any other) physicians in the United States. This article summarizes these two diseases as pulmonary manifestations of bioterrorism and discusses the possibility of avian influenza as a potential respiratory pathogen in biowarfare. It is hoped that phyisicians will need to know this information only as an academic exercise and not because of a clinical circumstance.

Introduction

The specter of biological warfare is no longer theoretical, nor has it been for many centuries. As reviewed by Christopher *et al.* [1] in a 1997 issue of the *Journal of the American Medical Association* devoted to the topic, as early as the 14th century, Tartar attackers catapulted plague-infected cadavers into a Ukrainian city to induce the disease. Likewise, Sir Jeffrey Amherst, the British commander of forces in North America during the French and Indian War (1754–1767), facilitated the spread of smallpox to Native Americans via blankets that had been used by smallpox victims.

Although a variety of infections are considered candidates for use in biological warfare (including smallpox, brucellosis, Q fever, tularemia, botulism, and staphylococcal enterotoxin disease [2]), two diseases—anthrax and plague—would present with respiratory symptoms. We discuss these infections in detail and outline the possibility of use of avian influenza in biowarfare.

Anthrax as a Biological Weapon

Anthrax, the first disease to fulfill Koch's postulates and the first bacterial disease for which immunization was available, may be the single greatest biological warfare threat [3]. A World Health Organization report estimated that 3 days after the release of 50 kg of anthrax spores along a 2-km line upwind of a city with a population of 500,000, 125,000 infections would occur and 95,000 people would die [4]. The US Congress Office of Technology Assessment estimated that between 130,000 and 3 million deaths would follow an aerosolized release of 100 kg of anthrax spores upwind of the Washington, DC area. This impact would exceed that of a hydrogen bomb [5]. Research on anthrax as a biological weapon began more than 80 years ago [1], and it has been thought that at least 17 nations have offensive biological weapons programs [6].

Microbiology

The word "anthrax" derives from Greek for coal, *anthracis,* because the disease causes black, coal-like skin lesions. Anthrax is caused by infection with *Bacillus anthracis*, a gram-positive spore-forming rod. The organism grows readily on all ordinary culture media at $37°C$; it has a "jointed bamboo-rod" cellular appearance and a unique "curled-hair" colonial appearance, and it displays no hemolysis on sheep agar. Anthrax spores germinate when entering an environment rich in amino acids, nucleosides, and glucose, such as that found in the blood or tissue of an animal or human host. Full virulence requires the presence of the antiphagocytic capsule and three toxin components (protective antigen, lethal factor, and edema factor). Vegetative bacteria have poor survival outside of animal or human hosts; in contrast, the environmentally hardy spore can survive for decades [7••]. Moreover, these spores, at 2 to 6 microns in diameter, are the ideal size for impinging on the mucosa of the human lower respiratory tract, optimizing the chance for infection [3].

Epidemiology and clinical manifestations

Endospores introduced into the body by abrasion, inhalation, or ingestion are phagocytosed by macrophages and carried to regional lymph nodes. They germinate inside the macrophages and become vegetative bacteria. The bacilli are then released from the macrophages, multiply in the lymphatic system, and enter the blood stream; there may be as many as 10^7 to 10^8 organisms per mL of blood, causing massive septicemia [8•]. This degree of bacteremia is many logs greater than in most clinical situations. Replicating bacteria release toxins that cause edema, hemorrhage, and necrosis. Three forms of anthrax are well recognized: inhalation, cutaneous, and gastrointestinal.

The inhalation form is particularly relevant to bioterrorism because in this form, anthrax spores are inhaled into the lungs and spread to the mediastinal lymph nodes, where germination takes place.

Inhalation anthrax

Germination is believed to occur for up to 60 days after exposure [9]. The process of delayed transformation of spores to vegetative cells is poorly understood but well documented. Disease occurs rapidly after germination takes place. In the famous outbreak of the disease in Sverdlovsk in the former Soviet Union, where anthrax spores had been accidentally released, cases occurred 2 to 43 days after the release [10]. However, viable spores have been found in the mediastinal lymph nodes of primates as long as 100 days after exposure [11]. The estimated dose in humans for 50% mortality is between 2.5×10^3 to 5.5×10^4 inhaled spores [7••].

Inhalation anthrax is characterized by hemorrhagic thoracic lymphadenitis and necrotizing mediastinitis. It is not a pneumonic process per se. Among the 42 cases in the Sverdlovsk outbreak that were examined postmortem, no cases of clinical pneumonia were noted; however, 11 cases had a focal necrotizing lung process thought to be analogous to a tuberculous Ghon complex [12]. About 50% of patients will also develop hemorrhagic meningitis as a terminal event.

Inhalation anthrax generally has two stages. The first part, which lasts hours or as long as a day or two, is an influenza-like illness with fever, weakness, headache, vomiting, chills, cough, and abdominal or chest pain [10]. The second, rapidly fulminating, stage involves high fever, dyspnea, diaphoresis, and shock. Chest radiography shows a widened mediastinum consistent with lymphadenopathy [13]. Patients generally rapidly deteriorate in hours, progressing from respiratory failure and shock to death.

In the United States, the mortality rate for inhalation anthrax was reported to be 89% [7••] in the first half of the 20th century, during which there were no intensive care units or, for the most part, antimicrobial agents. In the Sverdlovsk outbreak, 68 of 79 patients died [10]. The death rate was lower in patients who became ill more than 30 days after presumed exposure. Aggressive intensive care and early interventions might improve survival.

Cutaneous anthrax

This is the most common form of anthrax. It is most often observed in persons exposed to animal hides or wool that have been environmentally contaminated with spores. In the setting of bioterrorism with anthrax spores, cases of cutaneous disease may develop from aerosol spore contamination of the environment.

Small, painless macules or papules develop 3 to 5 days after endospores are introduced to subcutaneous tissue through cuts in the skin. These lesions become vesicular, with central necrosis surrounded by extensive local edema. The lesions dry and fall off in 1 to 2 weeks.

Antibiotic therapy does not change the course of cutaneous disease, but it is generally recommended to decrease the small but finite possibility of systemic disease. The mortality rate associated with cutaneous anthrax not treated with antibiotics can be as high as 20%. With proper antibiotic treatment, however, mortality is extremely low [14].

Gastrointestinal anthrax

In gastrointestinal anthrax, disease is acquired through ingestion of spore-contaminated meat products, such as cattle or sheep that have died of anthrax. In the upper gastrointestinal tract, common sites of involvement are the oral cavity and esophagus; in the lower tract, the terminal ileum and cecum are the most frequently affected. The oropharyngeal form is much milder; it is characterized by cervical edema, oral or esophageal pseudomembranous ulcerations, local lymphadenopathy leading to dysphagia, and respiratory difficulty.

In this form of anthrax, patients usually present with fever, diffuse abdominal tenderness, and bloody diarrhea. Two to 4 days later, ascites develops as abdominal pain decreases. Morbidity and death usually result from blood loss, electrolyte disturbances, intestinal perforation, and sepsis [15].

Diagnosis

Because anthrax is very rare, early cases should alert clinicians to a possible large epidemic. The sudden appearance of many patients in a city or region with an acute-onset influenza-like illness and case-fatality rates of 80% or more, with nearly half of all deaths occurring within 24 to 48 hours, is highly likely to represent an outbreak of anthrax or pneumonic plaque [7••].

Diagnosis of early anthrax is difficult because presentation often mimics that of other acute respiratory illnesses. A clue to the diagnosis of inhalation anthrax is a chest radiograph with a widened mediastinum in the setting of acute respiratory illness. The occurrence of these events in a previously healthy person should cause strong suspicion of inhalation anthrax.

The most useful microbiologic study is standard blood culture; results should be positive in 6 to 24 hours. If the clinical laboratory is alerted to the possibility of anthrax, it should be able to identify the organism in 12 to 24 hours. *B. anthracis* may be misidentified if the laboratory has not been alerted to that diagnosis. In diagnosis of inhalation anthrax, neither sputum culture nor Gram stain is helpful because the process is not pneumonia and the organism does not appear in the respiratory secretions.

Findings on postmortem examination should lead to diagnosis of inhalation anthrax if this diagnosis is not made before death. Thoracic hemorrhagic necrotizing lymphadenitis and hemorrhagic necrotizing mediastinitis in previously healthy persons are pathognomonic of inhalation

Table 1. Initial ciprofloxacin treatment or postexposure prophylaxis in patients with clinical inhalation anthrax*

Dosing for contained casualty	Treatment for mass casualty or postexposure dosing	Treatment duration
Adults: 400 mg IV every 12 h	Adults: 500 mg orally every 12 h	60 d for all regimens; change IV therapy to appropriate oral drug when appropriate
Children: 10–20 mg/kg (up to adult dose) IV every 12 h	Children: 10–15 mg/kg (up to adult dose) orally every 12 h	

Adapted from Inglesby *et al.* [7••].
*Equivalent doses of ofloxacin or levofloxacin may be used. The benefits of ciprofloxacin are likely to outweigh the risks in children and pregnant women.
IV—intravenously.

Table 2. Treatment or postexposure prophylaxis of clinical inhalation anthrax after microbiologic sensitivities are known

Dosing for contained casualty	Treatment for mass casualty or postexposure dosing	Treatment duration
Beta-lactam penicillins Penicillin G (adults and children ≥ 12 y): 4 million units IV every 4 h; children < 12 y: 50,000 units/kg IV every 6 h	Amoxicillin (adults, children ≥ 20 kg, and pregnancy): 500 mg orally every 8 h; children < 20 kg: 40 mg/kg/d in three doses	See Table 1
Tetracyclines Doxycycline* (adults and children > 45 kg): 100 mg IV every 12 h; children ≤ 45 kg: 2.5 mg/kg IV every 12 h	Doxycycline: 100 mg orally every 12 h	

Adapted from Inglesby *et al.* [7••].
*Equivalent doses of tetracycline may be used.
IV—intravenously.

anthrax [7••]. A cluster of such cases should alert public health authorities to the likelihood of aerosol spread of anthrax spores. In the absence of an accident in a nearby biowarfare weapons factory, this scenario is tantamount to the diagnosis of a bioterrorism event with anthrax.

Treatment
With the rapid course of symptomatic inhalation anthrax, early antibiotic therapy is essential. Given the difficulty in diagnosis, all persons with fever or evidence of systemic disease in an area where anthrax cases are occurring should be treated for anthrax until the disease is excluded [7••]. Even with antimicrobial intervention, the mortality rate is very high. Anthrax is usually sensitive to penicillin, but a *B. anthracis* vaccine strain engineered by Russian scientists reportedly resists both tetracycline and penicillin. It is recommended that ciprofloxacin or another quinolone be the first choice for therapy until the final sensitivity pattern is known, at which point the regimen can be adjusted accordingly. Tables 1 and 2 summarize the recommendations for treatment and prophylaxis of inhalation anthrax.

When used for prophylaxis in a presumed or proven setting of respiratory exposure to anthrax spores, therapy should last for at least 60 days because of the possibility of delayed germination. Delayed germination should also prompt a prolonged course of active therapy.

Infection control
Because no evidence suggests person-to-person transmission, no specific isolation is recommended [16]. High-efficiency particulate air filter masks and other measures for airborne protection are unnecessary after exposure to an affected patient. Extensive protocols are being considered, however, for infection control precautions for first responders to areas of possible aerosol contamination with anthrax spores. In the past few years, a significant number of "prank" exposures have occurred [17].

Vaccination
The anthrax virulence factors—lethal and edema factor—both depend on a protein called protective antigen for their toxic effects. In effect, protective antigen functions as the β-transport chain for the other toxins, facilitating their binding to and uptake into target cells. Protective antigen is the basis for the current US anthrax vaccine. The vaccine is an alum-adsorbed, partially purified culture filtrate of *B. anthracis* with a high content of protective antigen. It is given in six doses at 0, 2, and 4 weeks and 6, 12, and 18 months, with a yearly booster dose [18]. Human efficacy for licensure was supported by one single-blinded field study that reported 92.5% efficacy; however, most of the anthrax cases were cutaneous, not inhalation [19]. Although little information about the vaccine's utility in

preventing human inhalation anthrax is available, one primate study showed that inoculation with this vaccine at 0 and 2 weeks completely protected the recipients against an aerosol challenge [20].

A human live attenuated vaccine is produced and used in countries of the former Soviet Union. In western countries, however, live attenuated vaccines have been considered unsuitable for use in humans [7••].

The current US vaccine is given primarily only to military personnel because of concerns about the use of biological warfare during military conflict. The vaccine had been used for some personnel during the Persian Gulf conflict. Significant issues have arisen about the production, quality control, and safety of this product [21••]. Studies are investigating a less complicated regimen (*ie*, one that involves fewer doses) and a better method of mass production; these developments are necessary before vaccination on a larger scale, including to civilians, can be considered. A variety of newer vaccine candidates are being studied [20]. One in particular—a purified protective antigen combined with a lipid A–type adjuvant—appeared superior to the current vaccine in guinea pigs [22].

In persons who have been exposed to anthrax, vaccination may also be considered as an adjunct to antimicrobial prophylaxis because of the possibility for delayed germination [23].

Plague as a Biological Weapon

Plague is a zoonosis of rodents and their fleas caused by infection with *Yersinia pestis*, a gram-negative organism of the family Enterobacteriaceae. This zoonosis is endemic in a wide array of rodents and is spread among them and to humans by infected fleas. Plague is one of three diseases requiring quarantine (the others are cholera and yellow fever) that are subject to the World Health Organization International Health Regulations. Because of its high mortality rate (approximately 200 million deaths throughout history), infection with *Y. pestis* has attracted attention for development as a possible biological warfare agent.

Microbiology

Yersinia pestis is a pleomorphic, nonmotile, gram-negative coccobacillus that is facultatively anaerobic and grows readily on most culture media. The organism is nonfastidious and is infective to laboratory rodents. With Wayson or Giemsa stain, it shows characteristic bipolar staining, which gives it a characteristic "safety pin" appearance. It grows optimally at 28°C on blood agar, typically requiring 48 hours for observable growth, but colonies are initially much smaller than those of other Enterobacteriaceae and may be overlooked.

Yersinia pestis has distinctive virulence factors that are important for survival in mammalian and flea hosts. The genes encoding these virulence factors are carried by three plasmids. The first, Pst plasmid, is the smallest plasmid. Its genes encode a plasminogen activator protease that provides both fibrinolytic and coagulase activities. At 37°C, fibrinolysis is most active; at 28°C, coagulation predominates. This enzyme helps organisms grow and remain in flea guts or spread through tissues in mammals and bacteriocin (pesticin), which enhances iron uptake by *Y. pestis* in the mammalian host.

The second plasmid is low calcium response plasmid, and the third is the pFra plasmid. This contains the F1 envelope antigen (antiphagocytic during growth above 33°C) and the murine toxin. Other virulence factors act in concert with these, such that only 2% to 10% of the bacteria needed to cause death in mammals at 25°C are necessary at 37°C.

Epidemiology and clinical manifestations

Yersinia pestis is maintained in rodents and their fleas in both wild and domestic rodent cycles. Humans become infected from a flea bite when they intrude into the natural wild cycle or when domestic plague cycles become established. Plague transmission from person to person is a result of close exposure to patients with respiratory plague; this type of transmission is very rarely caused by direct skin or mucous membrane contact with infectious secretions or exudates. The transmission of infection from one person to another by fleas is controversial and, on the basis of epidemiologic studies, is considered rare.

The natural history of *Y. pestis* is complex and habitat-specific. More than 150 species of fleas and over 200 species of mammals have been found to be naturally infected with *Y. pestis*, although relatively few are important in maintaining enzootic or epizootic cycles and fewer still pose a significant risk to humans.

When a flea ingests a blood meal from a bacteremic animal infected with *Y. pestis*, the coagulase of the organism causes the blood to clot in the foregut, leading to blockage of the flea's swallowing. *Y. pestis* multiplies in the clotted blood. Such "blocked" fleas cannot digest their food and ultimately die. However, this state makes them ravenously hungry. In the fleas' attempt to feed, blood sucked from a mammalian host mixes with bacilli that are regurgitated back into the host.

In most cases of naturally occurring plague, the bite by a plague-infected flea leads to the inoculation of up to thousands of organisms into a patient's skin. The inoculated bacteria are engulfed by neutrophils and monocytes and migrate by cutaneous lymphatics to the regional lymph nodes, causing destruction and necrosis of lymph node architecture. Subsequent bacteremia, septicemia, and endotoxemia can quickly lead to shock, disseminated intravascular coagulation, and coma.

While the neutrophils can destroy bacilli, the monocytes cannot. *Y. pestis* resists destruction in mononuclear phagocytes and may multiply intracellularly with elaboration of the envelope antigen. If lysis of the mononuclear cell occurs, the bacilli released are

relatively resistant to further phagocytosis. Bacilli then multiply in lymph nodes and the blood and travel throughout the body, especially to the spleen, liver, lungs, and meninges [24].

Bubonic plague

After an incubation period of 2 to 8 days, affected individuals have sudden onset of fever, chills, weakness, and headache, followed by tender, erythematous lymphadenopathy, most commonly in the groin, axilla, or neck. "Bubo" is derived from the Greek "boubon," for groin. Buboes are 1 to 10 cm in diameter, and the overlying skin is erythematous. They are extremely tender, nonfluctuant, and warm and are often associated with considerable surrounding edema, but seldom lymphangitis. Buboes may point and drain spontaneously [25]. Bubo location is primarily a function of the region of the body in which an infected flea inoculates plague bacilli.

Plague is almost unique for the suddenness of onset of fever and bubo, the rapid development of intense inflammation in the bubo, and the fulminant clinical course that can produce death as quickly as 2 to 4 days after symptom onset.

Septicemic plague

A distinctive feature of plague is the propensity of the disease to overwhelm the patient with a massive growth of bacteria in the blood. The only other conditions in which bacteremia of this severity occurs are anthrax, meningococcemia, and postsplenectomy pneumococcemia. In the pathogenesis of plague infection, bacteria are sometimes inoculated and proliferate in the body without producing a bubo. Patients may become ill with fever and actually die with bacteremia but without detectable lymphadenitis. This syndrome has been termed "septicemic plague" to denote plague without a bubo. In New Mexico, 25% of plague was septicemic in 1980 to 1984, and the case-fatality rate in these cases (33%) was three times higher than in bubonic plague because of delays in diagnosis and treatment [26,27].

Pneumonic plague

Hematogenous spread of bacteria from the bubo to the lungs results in secondary pneumonia. It manifests in the setting of fever and lymphadenopathy as cough, chest pain, dyspnea, hemoptysis, and severe bronchopneumonia. In addition to the high mortality rate, plague pneumonia is highly contagious by airborne transmission. No chest radiographic pattern is characteristic of plague, but bilateral interstitial infiltrates are most commonly seen.

Plague after the use of a biological weapon

The pathogenesis and clinical manifestations of plague after a biological attack may be notably different from those seen after the usual naturally occurring plague. Inhaled aerosolized *Y. pestis* would cause primary pneumonic plague. The time of exposure to aerosolized plague bacilli until development of first symptoms in humans and nonhuman primates has been found to be 1 to 6 days and, most often, 2 to 4 days. The first sign of illness is expected to be fever with cough and dyspnea, sometimes with the production of bloody, watery, or, less commonly, purulent sputum [27–29]. Prominent gastrointestinal symptoms, including nausea, vomiting, abdominal pain, and diarrhea, may occur [30].

The ensuing clinical findings of primary pneumonic plague are similar to those of any severe rapidly progressive pneumonia and are those of secondary pneumonic plague. In contrast to secondary pneumonic plague, however, features of primary pneumonic plague would include the absence of buboes (except, rarely, cervical buboes) and, on pathologic examination, pulmonary disease with areas of profound lobular exudation and bacillary aggregation [31]. Chest radiographic findings vary, but bilateral infiltrates or consolidations are common.

Primary inhalation pneumonia is also a potential threat after exposure to a patient with plague who has a cough. The median infective inhaled dose is 100 to 500 bacilli [32]. However, only 1 to 10 bacilli can infect rodents or primates via the oral, intradermal, subcutaneous, or intravenous route [33].

Respiratory droplets can be inhaled by persons within 2 to 5 feet of production. In two cases of primary pneumonic plague contracted after the patients handled cats with pneumonic plague, both patients had pneumonic symptoms as well as prominent gastrointestinal symptoms (nausea, vomiting, abdominal pain, and diarrhea). Diagnosis and treatment were delayed more than 24 hours after symptom onset in both patients, both of whom died [34]. Plague pneumonia is invariably fatal when antibiotic therapy is delayed more than 1 day after the onset of illness [32].

Mucocutaneous manifestations

Patients with terminal pneumonic and septicemic plague, as would be seen in a biowarfare scenario, may develop livid cyanosis and large ecchymoses on the back. Septicemia could cause petechiae, purpura, ecchymoses, and acral necrosis [25,33]. The purpuric lesions may become necrotic, resulting in gangrene of the distal extremities, nose, and, in advanced disease, acral regions; this process is believed to be responsible for the medieval epithet "Black Death" [32].

Most patients with bubonic plague do not have skin lesions; however, about one fourth of the patients in Vietnam did have lesions [35]. Most common were pustules, vesicles, eschars, or papules near the bubo or in the anatomic region of skin that is lymphatically drained by the affected lymph nodes, presumably representing sites of the flea bites. Rarely, these skin lesions progress to extensive cellulitis or abscesses.

Plague can produce pharyngitis that may resemble acute tonsillitis associated with anterior cervical lymphadenopathy [36].

Meningitis
A rare complication, meningitis typically occurs more than 1 week after inadequately treated bubonic plague. It results from hematogenous spread from a bubo and carries a mortality rate higher than that seen with uncomplicated bubonic plague. Fever, headache, meningismus, pleocytosis, and a predominance of polymorphonuclear leukocytes are characteristic.

Diagnosis
The early diagnosis of plague requires a high index of suspicion in naturally occurring cases, especially after the possible use of a biological weapon. No effective environment warning symptoms are available to detect aerosolized plague bacilli [37].

The first indication of a clandestine terrorist attack would probably be a sudden outbreak of an illness presenting as severe pneumonia and hemoptysis. A fulminant course leading to azotemia, elevated aminotransferase levels, coagulation abnormalities, and evidence of multiorgan failure should suggest a diagnosis of plague.

There are no widely available rapid diagnostic tests for plague [37]. Tests that would be used to confirm suspected diagnoses are antigen detection, IgM enzyme immunoassay, immunostaining, and polymerase chain reaction.

These test are available only at some state health departments, the Centers for Disease Control and Prevention, and military laboratories. The routinely used passive hemagglutination antibody detection assay is typically only of retrospective value because several days to weeks usually pass after disease onset before these antibodies develop.

The leukocyte count is typically elevated, in the range of 10,000 to 20,000 cells/mm^3. Examination of the leukocytes in the peripheral blood reveals cytoplasmic vacuolations, toxic granulations, Dohle bodies, and gram-negative coccobacilli.

Microbiologic studies are important in the diagnosis of pneumonic plague. Stains and cultures of sputum, blood, bubo aspirates, cerebrospinal fluid, or even skin scrapings may be helpful in isolating the organism.

Treatment
Untreated plague has an estimated mortality rate of more than 50% in patients with bubonic disease and in nearly all patients with septicemic or pneumonic plague. Therefore, the early institution of effective antibiotic therapy is mandatory after appropriate cultures are obtained.

Recommendations for the use of antibiotics after exposure to plague from biological weapons are tempered by the lack of published trials on treating plague in humans, the limited number of studies in animals, and the possible requirement to treat a large number of persons.

For these reasons, the Working Group on Civilian Biodefense offers consensus recommendations based on the best available evidence. The recommendations do not necessarily represent uses currently approved by the Food and Drug Administration (FDA) or an official position on the part of any of the federal agencies whose scientists participated in these discussions [38••]. Recommendations will need to be revised as further relevant information becomes available.

Antibiotic therapy
Historically, streptomycin is the drug of choice and has been approved by the FDA for treatment of plague. Administered early during the disease, streptomycin has reduced overall plague mortality to 5% to 14%. Gentamicin is an acceptable substitute but is not FDA approved for this indication [26].

In a contained casualty setting, a situation in which a modest number of patients require treatment, the working group recommends parenteral antibiotic therapy (Table 3). However, in a mass casualty setting, intravenous or intramuscular therapy may not be possible, and parenteral therapy will need to be supplanted by oral therapy. Also for a mass casualty setting, the working group recommends oral therapy, preferably with doxycycline (or tetracycline) or ciprofloxacin (Table 4).

Clinical deterioration of patients despite early initiation of empiric therapy could signal antimicrobial resistance (which could have been genetically programmed by the bioterrorists) and should be promptly evaluated.

In general, antimicrobial therapy should be continued for 10 days, or for at least 3 days after the patient has become afebrile and has made a clinical recovery. Patients who had begun receiving intravenous antibiotics may be switched to oral regimens as indicated by clinical response.

Chloramphenicol is indicated for conditions in which high tissue penetration is important (*eg*, plague meningitis, pleuritis, endophthalmitis, and myocarditis).

Trimethoprim-sulfamethoxazole has been used successfully to treat bubonic plague but is not considered a first-line choice.

For patients allergic to streptomycin or those in whom an oral drug is strongly preferred, tetracycline is a satisfactory alternative. It is generally contraindicated in patients with renal failure, children younger than 7 years of age, and pregnant women.

Although in vitro testing has demonstrated the effectiveness of quinolones, rifampin, third-generation cephalosporins, and amoxicillin, these drugs have not been used extensively in human cases of plague [39,40].

Plague vaccine
The US-licensed formaldehyde-killed whole-bacilli vaccine was discontinued by its manufacturers in 1999 and is no longer available. Plans for future licensure and production are unclear [38••].

Table 3. Preferred treatment or postexposure prophylaxis for pneumonic plague

Treatment of contained casualty (10 days)	Treatment of mass casualty or postexposure prophylaxis (7 days)
Streptomycin: 1 g IM every 12 h (adults); 15 mg/kg (up to adult dose) IM every 12 h (children). Or, gentamicin: 5 mg/kg IM or IV every 24 h (adults) or 2 mg/kg loading dose then 1.7 mg/kg IM or IV every 8 h (adults, pregnancy); 2.5 mg/kg IM or IV every 8 h (children); 4 mg/kg loading dose then 2.5 mg/kg IM or IV every 12 h (neonates).	Doxycycline*: 100 mg orally bid (adults, pregnancy, children ≥ 45 kg); 2.2 mg/kg orally bid (children < 45 kg). Or, ciprofloxacin†: 500 mg orally bid (adults, pregnancy); 20 mg/kg (up to adult dose) orally bid (children).

Adapted from Inglesby *et al.* [38••].
*Or equivalent doses of tetracycline.
†Or equivalent doses of ofloxacin or levofloxacin.
bid—twice a day; IM—intramuscularly; IV—intravenously.

Table 4. Alternative treatment or postexposure prophylaxis for pneumonic plague

Treatment of contained casualty (10 days)	Mass casualty treatment or postexposure prophylaxis (7 days)
Doxycycline*: 100 mg IV every 12 h or 200 mg IV every 25 h (adults, pregnancy, children ≥ 45 kg); 2.2 mg/kg (up to adult dose) (children < 45 kg. Or, ciprofloxacin†: 400 mg IV every 12 h (adults, pregnancy); 15 mg/kg (up to adult dose) IV every 12 h (children). Or, chloramphenicol: 25 mg/kg IV every 6 h (adults, children ≥ 2 y).	Chloramphenicol: 25 mg/kg orally four times a day (not in children ≤ 2 y)

Adapted from Inglesby *et al.* [38••].
*Equivalent doses of tetracycline may be used.
†Equivalent doses of ofloxacin or levofloxacin may be used.

This killed vaccine demonstrated efficacy in preventing or ameliorating bubonic disease, but it did not clearly prevent or ameliorate the development of primary pneumonic plague [40,41]. The degree and duration of the immune response varied, and persons with continuing risk require monitoring of antibody levels. Adverse reactions after injection of the first dose were usually mild but can increase with repeated doses. The vaccine was given routinely to US troops during the Vietnam War. Evidence for efficacy was that the incidence of plague was lower in vaccinees than in unvaccinated South Vietnamese, and was lower than the incidence of murine typhus, another infection spread by the rodent flea [20].

Attempts at more immunogenic, live attenuated vaccines did not increase immunogenicity and caused some sporadic reversion of vaccine strains to virulent, wild-type bacteria [20]. The vaccine was also used in special circumstances for individuals deemed to be at high risk of developing plague, such as military personnel working in plague-endemic areas and persons employed in high-risk occupations (entomologists or laboratory workers using *Y. pestis*) [42].

Researchers continue to pursue a vaccine that protects against primary pneumonic plague [43]. For example, a multiple-subunit vaccine seemed to effectively protect mice against bubonic and pneumonic plague [44•].

Consideration for biological warfare with plague
The most common cutaneous manifestation of plague, the bubo, would not be present in a biological warfare scenario unless the Japanese plan (discussed below) of releasing infected fleas was resurrected.

While *Y. pestis* would most likely be aerosolized for a terrorism attack, the Japanese used a more creative approach in China during World War II. Human fleas (*Pulex irritans*) were infected with *Y. pestis*. These organisms were released into several Chinese cities, and small epidemics of plague ensued. Normally, animal hosts die in epizootics before humans are infected, but in these cases, humans died first and animals began dying of plague [45,46].

In a terrorist attack, plague would most likely be transmitted as an aerosol [2]. It should be noted, however, that environmental factors can affect aerosolization of a vegetative agent (bacteria or virus) as compared to a spore in an outside environment. Such attacks would most likely be successful late at night or early in the morning, when the agents would be less likely to undergo inactivation by ultraviolet radiation. Also at such times, atmospheric temperature inversions could allow an agent cloud to travel at low altitude to cover its target.

The possibility of rapid death, combined with possible person-to-person transmission (in contrast to anthrax), makes plague an ominous biological warfare

threat. The United States studied *Y. pestis* as a potential offensive weapon in the 1950s. Many other countries are also suspected of developing plans to use plague as a biological weapon [2].

Avian Influenza as a Biological Weapon

In 1997, a large outbreak of influenza occurred in chickens in Hong Kong [47]. The organism involved was found to be an H5N1 strain. However, because it is generally accepted that only hemagglutinin types 1, 2, and 3 are pathogenic to humans (although anecdotal exceptions have been reported), the risk to humans was thought to be negligible. The widespread slaughter of poultry in Hong Kong seemed to avert further spread in fowl, but the organism has continued to circulate to some degree in Asia [48].

Of even more concern to human epidemiology were 18 cases of infection with influenza H5N1 in humans; patients were significantly ill, and six relatively young people died of respiratory failure. These deaths suggested that the causative organism or one similar might produce an influenza pandemic like the one in 1918–1920, which was due to H1N1. Fortunately, it was apparent that all the overt human infections were related to direct fowl exposure, not from human-to-human spread or eating or preparing poultry products [49]. That some human-to-human transmission did occur is clear, however, a small but finite seropositive rate (3.7%, 8 of 217) was higher in health care workers exposed to infected people than in nonexposed health care workers (0.7%, 2 of 309). This finding was significant even after adjustment for poultry exposure [50]. Secondary human-to-human transmission did occur, therefore, but was not clinically significant.

Of note, standard techniques for producing influenza vaccine (in eggs) were not successful with this virus because it was very virulent in the eggs. Alternative, newer technology vaccines were successful (genomic vaccine, baculovirus vector vaccines) in poultry models.

This naturally occurring epidemic illustrates the possibility that similar avian influenza strains could function as biological warfare agents. They would need to be highly virulent in infected humans on primary human passage (as the H5N1 was), would need to be as efficiently transmitted from person to person as standard strains (via aerosols or direct contact), and would need to be as virulent in secondary cases. Such a new agent could not only cause substantial numbers of primary influenzal pneumonia deaths but also contribute to morbidity and death from secondary bacterial pneumonia while a prototypic vaccine could be produced. It may be possible to genetically engineer such viruses to resist amantadine and the newer neuraminidase inhibitors.

Conclusions

Although the risk of a nuclear war has been substantially lessened since the end of the Cold War, the parallel risk that a rogue nation or terrorist group would use biological warfare has increased. This increase is also related to the dissolution of the Soviet Union. This dichotomy of risk is related to the ease of setting up laboratories for biological work and the even easier transport of pathogens compared with the effort needed to produce nuclear weapons. When the Soviet Union dissolved, the extensive biowarfare industry set up by the Russians is likely to have disseminated to eagerly waiting rogue nations and terrorist groups via unpaid and unhappy scientists.

The extent of the biological warfare work in turning pathogens into weapons in the former Soviet Union has been documented in Ken Alibek's *Biohazard* [51••]. Alibek was a high-level scientist in the program until he defected to the West. The degree of work in biowarfare appears to have been grossly underestimated by western nations. Books such as *The Cobra Event* [52] and *The Eleventh Plague* [53] are works of fiction. May they stay that way.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Christopher GW, Cieslak TJ, Pavlin JA, *et al.*: **Biological warfare. A historical perspective.** *JAMA* 1997, **278**:412–417.
2. Franz DR, Jahrling PB, Friedlander AM, *et al.*: **Clinical recognition and management of patients exposed to biological warfare agents.** *JAMA* 1997, **278**:399–411.
3. Cieslak TJ, Eitzen EM Jr: **Clinical and epidemiologic principles of anthrax.** *Emerg Infect Dis* 1999, **5**:552–555.
4. WHO groups of consultants: *Health Aspects of Chemical and Biological Weapons.* Geneva: World Health Organization; 1970:97–99.
5. Simon JD: **Biological terrorism: preparing to meet the threat.** *JAMA* 1997, **278**:428–430.
6. Cole LA: **The specter of biological weapons.** *Sci Am* 1996, **275**:60–65.
7.•• Inglesby TV, Henderson DA, Barlett JG, *et al.*: **Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense.** *JAMA* 1999, **281**:1735–1745.
One of the three (as of this writing) summary statements from the Working Group on Civilian Biodefense. These excellent and up-to-date documents will help clinicians recognize and understand each condition discussed.
8.• Dixon TC, Meselson M, Guillemin J, *et al.*: **Anthrax.** *N Engl J Med* 1999, **341**:815–826.
A well-written summary on anthrax that will complement the biowarfare literature.
9. Friedlander A, Welkos SL, Pitt ML, *et al.*: **Postexposure prophylaxis against experimental inhalation anthrax.** *J Infect Dis* 1993, **167**:1239–1242.
10. Meselson M, Guillemin J, Hugh-Jones M, *et al.*: **The Sverdlovsk anthrax outbreak of 1979.** *Science* 1994, **266**:1202–1208.
11. Henderson DW, Peacock S, Belton FC: **Observations on the prophylaxis of experimental pulmonary anthrax in the monkey.** *J Hygiene* 1954,**54**:28–36.

12. Aramova FA, Grinberg LM, Yampolskaya O: **Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak in 1979.** *Proc Natl Acad Sci U S A* 1993, **90:**2291–2294.

13. Vessal K, Yeganehdoust J, Duttz W, *et al.*: **Radiologic changes in inhalation anthrax.** *Clin Radiol* 1975, **26:**471–474.

14. Lew D: **Bacillus anthracis (anthrax).** In *Principles and Practices of Infectious Disease.* Edited by Mandell GL, Bennett JE, Dolin R. New York: Churchill Livingstone; 2000:2215–2220.

15. Alizad A, Ayoub EM, Makki N: **Intestinal anthrax in a two year old child.** *Pediatr Infect Dis J* 1995, **14:**394– 395.

16. Pile JC, Malone JD, Eitzen EM, *et al.*: **Anthrax as a potential biological warfare agent.** *Arch Intern Med* 1998, **158:**429–434.

17. Stern J: **The prospect of domestic bioterrorism.** *Emerg Infect Dis* 1999, **5:**517–522.

18. Cieslak TJ, Christopher GW, Kortepeter MG, *et al.*: **Immunization against potential biological warfare agents.** *Clin Infect Dis* 1999, **30:**843–850.

19. Brachman PS, Gold H, Plotkin SA, *et al.*: **Field evaluation of a human anthrax vaccine.** *Am J Pub Health* 1962, **52:**632–645.

20. Russell PK: **Vaccine in civilian defense against bioterrorism.** *Emerg Infect Dis* 1999, **5:**531–533.

21.•• Nass M: **Anthrax vaccine: a model of a response to the biologic warfare threat.** *Infect Dis Clin North Am* 1999, **13:**187–208.
Well-organized exposé on anthrax vaccine and its implications for the military-biological complex.

22. Ivins B, Fellows P, Pitt L, *et al.*: **Experimental anthrax vaccines: efficacy of adjuvants combined with protective antigen against an aerosol Bacillus anthracis spore challenge in guinea pigs.** *Vaccine* 1995, **13:**1779–1784.

23. **Bioterrorism alleging use of anthrax and interim guidelines for management-United States, 1998.** *MMWR Morbid Mortal Wkly Rep* 1999, **48:**69–74.

24. Perry RD, Fetherston JD: **Yersinia pestis: etiologic agent of plague.** *Clin Microbiol Rev* 1997, **10:**35–66.

25. Welty TK, Grabman J, Kompare E: **Nineteen cases of plague in Arizona: a spectrum including ecthyma gangrenosum due to plague and plague in pregnancy.** *West J Med* 1985, **142:**641–646.

26. Hull HF, Montes JM, Mann JM: **Septicemic plague in New Mexico.** *J Infect Dis* 1987, **155:**113–118.

27. Crook LD, Tempest B: **Plague: a clinical review of 27 cases.** *Arch Intern Med* 1992, **152:**1253–1256.

28. Speck RS, Wolochow H: **Studies on the experimental epidemiology of respiratory infections, VIII: Experimental pneumonic plague in Macacus rhesus.** *J Infect Dis* 1957, **100:**58–68.

29. Wu L-T: *A Treatise on Pneumonic Plague.* Geneva, Switzerland: League of Nations Health Organization; 1926.

30. Poland JD, Dennis DT: **Plague.** In *Bacterial Infections of Humans: Epidemiology and Control.* Edited by Evans AS, Brachman PS. New York: Plenum Medical Book; 1998:545–558.

31. Dennis D, Meier F: **Plague.** In *Pathology of Emerging Infections.* Edited by Horsburgh CR, Nelson AM. Washington, DC: ASM Press; 1997:21–47.

32. Brubaker RR: **Factors promoting acute and chronic diseases caused by Yersiniae.** *Clin Microbiol Rev* 1991;4:309–324.

33. Cavanaugh DC, Cadigan FC, Williams JE, *et al.*: **Plague.** In *General Medicine and Infectious Diseases.* Edited by Ognibene AJ, Barrett ON. Washington, DC: Office of The Surgeon General and Center of Military History; 1982.

34. Werner SB, Weidmer CE, Nelson BC, *et al.*: **Primary plague pneumonia contracted from a domestic cat in South Lake Tahoe, California.** *JAMA* 1984, **251:**929–931.

35. Legters LJ, Cottingham AJ Jr, Hunter DH: **Clinical and epidemiologic notes on a defined outbreak of plague in Vietnam.** *Am J Trop Med Hygiene* 1970, **19:**639–652.

36. Cleri DJ, Vernaleo JR, Lombardi LJ, *et al.*: **Plague pneumonia disease caused by Yersinia pestis.** *Sem Respir Infect* 1997, **12:**12–23.

37. Institute of Medicine National Research Council: **Detection and measurement of biological agents.** In *Chemical and Biological Terrorism: Research and Development to Improve Civilian Medical Response.* Washington, DC: National Academy Press; 1999:95.

38.•• Inglesby TV, Dennis DT, Henderson DA, *et al.*: **Plague as a biologic weapon: medical and public health management.** *JAMA* 2000, **283:**2281–2290.
This complementary article to Inglesby *et al.* [7••] covers plague quite well.

39. McGovern TW, Friedlander A: **Plague.** In *Medical Aspects of Chemical and Biological Warfare.* Edited by Sidell FR, Takafuji ET, Franz DR. Falls Church, VA: Office of the Surgeon General, United States Army; 1997:479–502.

40. Russell P, Eley SM, Bell DL, *et al.*: **Doxycycline or ciprofloxacin prophylaxis and therapy against experimental Yersinia pestis infection in mice.** *J Antimicrob Chemother* 1996, **37:**769–774.

41. Ehrenkranz NF, Meyer KF: **Studies on immunization against plague, VIII: Study of three immunizing preparations in protecting primates against pneumonic plague.** *J Infect Dis* 1955, **96:**138–144.

42. Meyer KF, Cavanaugh DC, Bartelloni PJ, *et al.*: **Plague immunization, I: Past and present trends.** *J Infect Dis* 1974, **129(Suppl):**S13–S18.

43. Titball RW, Eley S, Williamson ED, *et al.*: **Plague.** In *Vaccines.* Edited by Plotkin S, Mortimer EA. Philadelphia: WB Saunders; 1999:734–742.

44.• Heath DG, Anderson GW, Mauro JM, *et al.*: **Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1-V antigen fusion protein vaccine.** *Vaccine* 1998, **16:**1131– 1137.
A good illustrative article on the application of new vaccine technologies.

45. Williams P, Wallace D: *Unit 731: Japan's Secret Biological Warfare in World War II.* New York: Free Press; 1989.

46. Harris S: **Japanese biological warfare research on humans: a case study of microbiology and ethics.** *Ann N Y Acad Sci* 1992, **555:**21–52.

47. Shortridge KF, Gao P, Guan Y, *et al.*: **Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective.** *Vet Microbiol* 2000, **74:**141–147.

48. Cauthen AN, Swayne DE, Schultz-Cherry S, *et al.*: **Continued circulation in China of highly pathogenic avian influenza viruses encoding the hemagglutinin gene associated with the 1997 H5N1 outbreak in poultry and humans.** *J Virol* 2000, **74:**6592–6599.

49. Mounts AW, Kwong H, Izurieta HS, *et al.*: **Case-control study of risk factors for avian influenza A (H5N1) disease, Hong Kong, 1997.** *J Infect Dis* 1999, **180:**505–508.

50. Buxton Bridges C, Katz JM, Seto WH, *et al.*: **Risk of influenza A (H5N1) infection among health care workers exposed to patients with influenza A (H5N1), Hong Kong.** *J Infect Dis* 2000, **181:**344–348.

51.•• Alibek KW: *Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World.* New York: Random House; 1999.
If you want to miss a few nights' sleep, read this book on the biowarfare industry in the former Soviet Union.

52. Preston R: *The Cobra Event.* New York: Random House; 1997.

53. Marr JS, Baldwin J: *The Eleventh Plague.* New York: Harper Trade; 1998.