

Biological Threat Agents

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Anthrax

Anthrax is a zoonotic disease caused by *Bacillus anthracis*. There are three types of this disease: cutaneous anthrax, inhalation anthrax, and gastrointestinal anthrax. About 95% of the human anthrax cases in the United States have been in the former category. Cutaneous anthrax develops when a bacterial organism from infected animal tissues becomes deposited under the skin. When a patient contracts cutaneous anthrax, he develops a small elevated lesion on his skin which becomes a skin ulcer, frequently surrounded by swelling or edema. The lymph gland near the lesion may also swell from the infection. If the lesion occurs on the neck or on or about the eye, it may cause complications. The incubation period for cutaneous anthrax is from one to seven days. When a patient does not receive an effective antibiotic, the mortality rate for cutaneous anthrax is 10-20%. With treatment, the mortality rate falls to less than 1%.

Inhalation anthrax develops when the bacterial organism is inhaled into the lungs. A progressive infection follows. Since inhalation anthrax is usually not diagnosed in time for treatment, the mortality rate in the United States is 90-100%. A biological attack with anthrax spores delivered by aerosol would cause inhalation anthrax, an extraordinarily rare form of the naturally occurring disease.

A lethal dose of anthrax is considered to be 10,000 spores; 80 percent of a population that inhaled such a dose would die. Less than one millionth of a gram is invariably fatal within five days to a week after exposure. According to an estimate by the U.S. Congress's Office of Technology Assessment, 100 kilograms of anthrax, released from a low-flying aircraft over a large city on a clear, calm night, could kill one to three million people.

The disease begins after an incubation period varying from 1-6 days, presumably dependent upon the dose of inhaled organisms. Onset is gradual and nonspecific, with fever, malaise, and fatigue, sometimes in association with a nonproductive cough and mild chest discomfort. In some cases, there may be a short period of improvement. The initial symptoms are followed in 2-3 days by the abrupt development of severe respiratory distress with dyspnea, diaphoresis, stridor, and cyanosis. Physical findings may include evidence of pleural effusions, edema of the chest wall, and meningitis. Chest x-ray reveals a dramatically widened mediastinum, often with pleural effusions, but typically without infiltrates. Shock and death usually follow within 24-36 hours of respiratory distress onset.

An epidemic of inhalation anthrax in its early stage with nonspecific symptoms could be confused with a wide variety of viral, bacterial, and fungal infections. Progression over 2-3 days with the sudden development of severe respiratory distress followed by shock and death in 24-36 hours in essentially all untreated cases eliminates diagnoses other than inhalation anthrax. The presence of a widened mediastinum on chest x-ray, in particular, should alert one to the diagnosis. Other suggestive findings include chest-wall edema, hemorrhagic pleural effusions, and hemorrhagic meningitis. Other diagnoses to consider include aerosol exposure to SEB; but in this case onset would be more rapid after exposure (if known), and no prodrome would be evident prior to onset of severe respiratory symptoms. Mediastinal widening on chest x-ray will also be absent. Patients with plague or tularemia pneumonia will have pulmonary infiltrates and clinical signs of pneumonia (usually absent in anthrax).

Almost all cases of inhalation anthrax in which treatment was begun after patients were symptomatic have been fatal, regardless of treatment. Historically, penicillin has been regarded as the treatment of choice, with 2 million units given intravenously every 2 hours. Tetracycline and erythromycin have been recommended in penicillin-sensitive patients. The vast majority of anthrax strains are sensitive in vitro to penicillin. However, penicillin-resistant strains exist naturally, and one has been recovered from a fatal human case. Moreover, it is not difficult to induce resistance to penicillin, tetracycline, erythromycin, and many other antibiotics through laboratory manipulation of organisms. All naturally occurring strains tested to date have been sensitive to erythromycin, chloramphenicol, gentamicin, and ciprofloxacin.

Vaccines are available against some forms of anthrax, but their efficacy against abnormally high concentrations of the bacteria is uncertain. A licensed, alum-precipitated preparation of purified *B.anthraxis* protective antigen (PA) has been shown to be effective in preventing or significantly reducing the incidence of inhalation anthrax. Limited human data suggest that after completion of the first three doses of the recommended six-dose primary series (0, 2, 4 weeks, then 6, 12, 18 months), protection against both cutaneous and inhalation anthrax is afforded. As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose; vaccine-induced protection is undoubtedly overwhelmed by extremely high spore challenge.

If there is information indicating that a biological weapon attack is imminent, prophylaxis with ciprofloxacin (500 mg orally twice a day), or doxycycline (100 mg orally twice a day) is recommended. If unvaccinated, a single 0.5 mL dose of vaccine should also be given subcutaneously. Should the attack be confirmed as anthrax, antibiotics should be continued for at least 4 weeks in all exposed.

Botulinum Toxin

Botulism is caused by intoxication with any of the seven distinct neurotoxins produced by the bacillus, *Clostridium botulinum*. In humans, disease results from only four (A, B, E, and F) of those seven types of neurotoxins. The toxins are proteins with molecular weights of approximately 150,000, which bind to the presynaptic membrane of neurons at peripheral cholinergic synapses to prevent release of acetylcholine and block neurotransmission. The blockade is most evident clinically in the cholinergic autonomic nervous system and at the neuromuscular junction. A biological attack with botulinum toxin delivered by aerosol would be expected to cause symptoms similar in most respects to those observed with food-borne botulism.

In pure form, the toxin is a white crystalline substance that is readily dissolvable in water, but decays rapidly in the open air. Symptoms of inhalation botulism may begin as early as 12-36 hours following exposure or as late as several days. Initial signs and symptoms include ptosis, generalized weakness, lassitude, and dizziness. Diminished salivation with extreme dryness of the mouth and throat may cause complaints of a sore throat. Urinary retention or ileus may also occur. Motor symptoms usually are present early in the disease; cranial nerves are affected first with blurred vision, diplopia, ptosis, and photophobia. Development of respiratory failure may be abrupt. Mucous membranes of the mouth may be dry and crusted.

Neurological examination shows flaccid muscle weakness of the palate, tongue, larynx, respiratory muscles, and extremities. Deep tendon reflexes vary from intact to absent.

The occurrence of an epidemic with large numbers of afebrile patients with progressive ocular, pharyngeal, respiratory, and muscular weakness and paralysis hints strongly at the diagnosis. Single cases may be confused with various neuromuscular disorders such as atypical Guillain-Barré syndrome, myasthenia gravis, or tick paralysis. The edrophonium (tensilon) test may be transiently positive in botulism.

Respiratory failure secondary to paralysis of respiratory muscles is the most serious complication and, generally, the cause of death. Reported cases of botulism prior to 1950 had a mortality of 60%. With tracheotomy and ventilator assistance, fatalities should be <5%. Intensive and prolonged nursing care may be required for recovery (which may take several weeks or even months).

A pentavalent toxoid of *Clostridium botulinum* types A, B, C, D, and E is available under IND status. This product has been administered to several thousand volunteers and occupationally at-risk workers and induces serum antitoxin levels that correspond to protective levels in experimental animal systems. The currently recommended schedule (0, 2, and 12 weeks, then a 1 year booster) induces solidly protective antitoxin levels in greater than 90 percent of those vaccinated after 1 year.

Brucellosis

Brucellosis is a systemic zoonotic disease that, in humans, is caused by one of four species of bacteria: *Brucella melitensis*, *B. abortus*, *B. suis*, and *B. canis*; virulence for humans decreases somewhat in the order given. These bacteria are small gram-negative, aerobic, non-motile coccobacilli that grow within monocytes and macrophages. They reside quiescently in tissue and bone-marrow, and are extremely difficult to eradicate even with antibiotic therapy. Their natural reservoir is domestic animals, such as goats, sheep, and camels (*B. melitensis*); cattle (*B. abortus*); and pigs (*B. suis*). *Brucella canis* is primarily a pathogen of dogs, and only occasionally causes disease in humans upon contact with an infected dog's blood, semen, or placenta. Humans are infected when they ingest raw (unpasteurized), infected milk or meat, inhale contaminated aerosols, or have abraded skin or conjunctival surfaces that come in contact with the bacteria. Laboratory infections are quite common, but human-to-human transmission is rare and occurred through breastfeeding. Therefore, isolation of infected patients is not required. *Brucella* species long have been considered potential candidates for use in biological warfare. The organisms are readily lyophilized, perhaps enhancing their infectivity. Under selected environmental conditions (for example, darkness, cool temperatures, high CO₂), persistence for up to 2 years has been documented. When used as a biological warfare agent, *Brucellae* would most likely be delivered by the aerosol route; the resulting infection would be expected to mimic natural disease.

Brucellosis presents after an incubation period normally ranging from 3-4 weeks, but may be as short as 1 week or as long as several months. Clinical disease presents typically as an acute, non-specific febrile illness with chills, sweats, headache, fatigue, myalgias, arthralgias, and anorexia. Cough occurs in 15-25%, but the chest x-ray usually is normal. Complications include sacroiliitis, arthritis, vertebral osteomyelitis, epididymo-orchitis, and rarely endocarditis. Physical findings include lymphadenopathy in 10-20% and splenomegaly in 20-30% of cases. Untreated disease can persist for months to years, often with relapses and remissions. Disability may be pronounced. Lethality may approach 6% following infection with *B. melitensis*, but the disease is rarely fatal (0.5% or less) after infection with other serotypes (usually after endocarditis develops).

The initial symptoms of brucellosis are usually nonspecific, and the differential diagnosis is therefore very broad and includes bacterial, viral, and mycoplasmal infections. The systemic symptoms of viral and mycoplasmal illnesses, however, are usually present for only a few days, while they persist for prolonged periods in brucellosis. Brucellosis may be

indistinguishable clinically from the typhoidal form of tularemia or from typhoid fever itself. The disease in humans is characterized by a multitude of somatic complaints, including fever, sweats, anorexia, fatigue, malaise, weight loss, and depression. Localized complications may involve the cardiovascular, gastrointestinal, genitourinary, hepatobiliary, osteoarticular, pulmonary and nervous systems. Without adequate and prompt antibiotic treatment, some patients develop a 'chronic' brucellosis syndrome with many features of the 'chronic fatigue' syndrome.

The recommended treatment is doxycycline (200 mg/day) plus rifampin (900 mg/day) for 6 weeks. Alternative effective treatment consists of doxycycline (200 mg/day) for 6 weeks plus streptomycin (1 gm/day) for 3 weeks. Trimethoprim-sulfamethoxazole given for 4-6 weeks is less effective. In 5-10% of cases, there may be relapse or treatment failure. Laboratory infections with brucellosis are quite common, but there is no human-to-human transmission and isolation is not required.

Killed and live attenuated human vaccines of unproven efficacy have been available in many countries, but not in the U.S. and many parts of Europe. There is no information on the use of antibiotics for prophylaxis against human brucellosis.

Cholera

Cholera is a diarrheal disease caused by *Vibrio cholera*, a short, curved, gram-negative bacillus. Humans acquire the disease by consuming water or food contaminated with the organism. The organism multiplies in the small intestine and secretes an enterotoxin that causes a secretory diarrhea. When employed as a BW agent, cholera will most likely be used to contaminate water supplies. It is unlikely to be used in aerosol form. Without treatment, death may result from severe dehydration, hypovolemia and shock. Vomiting is often present early in the illness and may complicate oral replacement of fluid losses. There is little or no fever or abdominal pain.

Watery diarrhea can also be caused by enterotoxigenic *E. coli*, rotavirus or other viruses, noncholera *Vibrios*, or food poisoning due to ingestion of preformed toxins such as those of *Clostridium perfringens*, *Bacillus cereus*, or *Staphylococcus aureus*.

Treatment of cholera depends primarily on replacement of fluid and electrolyte losses. This is best accomplished using oral dehydration therapy with the World Health Organization solution (3.5 g NaCl, 2.5 g NaHCO₃, 1.5 g KCl and 20 g glucose per liter). Intravenous fluid replacement is occasionally needed when vomiting is severe, when the volume of stool output exceeds 7 liters/day, or when severe dehydration with shock has developed. Antibiotics will shorten the duration of diarrhea and thereby reduce fluid losses.

Improved oral cholera vaccines are presently being tested. One vaccine, which has been discontinued in the U.S., is a killed suspension of *V. cholera* provided about 50% protection that lasts for no more than 6 months. The initial dose is two injections given at least 1 week apart with booster doses every 6 months.

***Clostridium Perfringens* Toxin**

Clostridium perfringens is a common anaerobic bacterium associated with three distinct disease syndromes; gas gangrene or clostridial myonecrosis; enteritis necroticans (pig-bel); and perfringens food poisoning. Each of these syndromes has very specific requirements for delivering inocula of *C. perfringens* to specific sites to induce disease, and it is difficult to imagine a general scenario in which the spores or vegetative organisms could be used as a biological threat agent. There are, however, at least 12 protein toxins elaborated, and one or more of these could be produced, concentrated, and used as a weapon. Waterborne disease is conceivable, but unlikely. The alpha toxin would be lethal by aerosol. This is a well characterized, highly toxic phospholipase C. Other toxins from the organism might be co-weaponized and enhance effectiveness. For example, the epsilon toxin is neurotoxic in laboratory animals.

Gas gangrene is a well-recognized, life-threatening emergency. Symptoms of the disease may be subtle before fulminant toxemia develops, and the diagnosis is often made at postmortem examination. The bacteria produce toxins that create the high mortality from clostridial myonecrosis, and which produce the characteristic intense pain out of proportion to the wound. Within hours, signs of systemic toxicity appear, including confusion, tachycardia, and sweating. Most *Clostridia* species produce large amounts of CO₂ and hydrogen that cause intense swelling, hence the term "gas" gangrene, resulting in gas in the soft tissues and the emission of foul-smelling gas from the wound. Clinical features include necrosis, dark red serous fluid, and numerous gas filled vesicles. The infection may progress up to 10 cm per hour, and early diagnosis and therapy are essential to prevent rapid progression to toxemia and death. Pulmonary findings might lead to confusion with staphylococcal enterotoxin B (SEB) initially. Liver damage, hemolytic anemia, and thrombocytopenia are not associated with SEB and the pulmonary findings should be reversible in SEB.

No specific treatment is available for *C. perfringens* intoxication. Early antibiotic treatment is effective, if undertaken before significant amounts of toxins have accumulated in the body. If not treated the bacteria enter the bloodstream causing fatal systemic illness. The organism itself is sensitive to penicillin, and consequently, this is the current drug of choice. Recent data indicate that clindamycin or rifampin may suppress toxin production and provide superior results in animal models. Prompt surgical debridement and broad spectrum, intravenous antibiotics are the mainstay of therapy. Hyperbaric oxygen has been seen as effective in prolonging survival in animal studies when coupled with other treatments.

There is no available prophylaxis against most *C. perfringens* toxins. Toxoids are being used to prevent enteritis necroticans in humans, and veterinary toxoids are in wide use.

Crimean-Congo Hemorrhagic Fever

Crimean-Congo hemorrhagic fever (CCHF) is a viral disease caused by the *Nairovirus*. The virus, first characterized in the Crimea in 1944, is transmitted by ticks, principally of the genus *Hyalomma*, with intermediate vertebrate hosts varying with tick species. In 1969 it was recognized that the pathogen causing Crimean hemorrhagic fever was the same as that responsible for an illness identified in 1956 in the Congo, and linkage of the two place-names resulted in the current name for the disease and the virus. The disease, next found in the Congo, occurs also in the Middle East, the Balkans, the former USSR, and eastern China. Little is known about variations in the virus properties over the huge geographic area involved. Humans become infected through tick bites, crushing an infected tick, or at the slaughter of viremic livestock. Even in epidemics, cases do not show narrow clustering and person-to-person spread is rare, though possible through contact with infectious blood or bodily fluids. CCHF would probably be delivered by aerosol if used as a BW agent.

The length of the incubation period for illness appears to depend on the mode of acquisition of the virus. Following infection via tick bite, the incubation period is usually one to three days, with a maximum of nine days. The incubation period following contact with infected blood or tissues is usually five to six days, with a documented maximum of 13 days. Typical cases present with sudden onset of fever and chills 3-12 days after tick exposure. There is severe headache, lumbar pain, nausea and vomiting, delirium, and prostration. Fatal cases are associated with extensive hemorrhage, coma, and shock. Mortality among cases recognized as hemorrhagic fever is 15-30%, with death occurring in the second week of illness. In those patients who recover, improvement generally begins on the ninth or tenth day after onset of illness. Convalescence in survivors is prolonged with asthenia, dizziness, and often hair loss.

Diagnosis of suspected CCHF is performed in specially-equipped, high biosafety level laboratories. Other viral hemorrhagic fevers, meningococemia, rickettsial diseases, and similar conditions may resemble full-blown CCHF. Most fatal cases and half the others will have detectable antigen by rapid enzyme-linked immunosorbant assay (ELISA) testing of acute serum samples. IgM ELISA antibodies occur early in recovery. Polymerase chain reaction has recently been used in diagnosing CCHF

Supportive therapy with replacement of clotting factors is indicated. Crimean-Congo hemorrhagic fever virus is sensitive to ribavirin *in vitro* and clinicians have been favorably impressed in uncontrolled trials. Immune sera have also been used for therapeutic purposes several times, but its value has not been demonstrated.

When patients with CCHF are admitted to the hospital, there is a risk of nosocomial spread of infection. In the past, serious outbreaks have occurred in this way and it is imperative that adequate infection control measures be observed to prevent this disastrous outcome. Patients with suspected or confirmed CCHF should be isolated and cared for using barrier nursing techniques. Because of several well-defined outbreaks within hospitals, protective measures for medical personnel are an issue. The weight of evidence points to large droplets or fomites as the mediators of transmission and so strict barrier nursing is indicated and probably sufficient for the care of naturally acquired disease. The virus is aerosol-infectious and additional precautions (for example, respirators) might be considered in a biological warfare setting.

Although there is little field experience and no definitive data on efficacy, the sensitivity of the virus to ribavirin and the severity of disease suggest that prophylaxis of high-risk exposures is indicated. In the case of a suspected biological attack, ribavirin could be considered for prophylaxis, but there is insufficient information to make a firm recommendation for dosing. An inactivated mouse-brain vaccine is used on a small scale in Eastern Europe, but there is no safe and effective vaccine widely available for human-use.

Ebola Hemorrhagic Fever

Ebola hemorrhagic fever is one of the most virulent viral diseases known to humankind, causing death in 50-90% of all clinically-ill cases. Consequently, it has figured prominently in popular discussions of biological weapons, although its practical applications as a biological threat agent remain speculative. The disease has its origins in the jungles of Africa and Asia and several different forms of Ebola virus have been identified and may be associated with other clinical expressions, on which further research is required.

The Ebola virus is transmitted by direct contact with the blood, secretions, organs or semen of infected persons. Transmission through semen may occur up to 7 weeks after clinical recovery, as with Marburg hemorrhagic fever. Health care workers have frequently been infected while attending patients. In the 1976 epidemic in Zaire, every Ebola case caused by contaminated syringes and needles died.

After an incubation period of 2 to 21 days, Ebola is often characterized by the sudden onset of fever, weakness, muscle pain, headache and sore throat. This is followed by vomiting, diarrhea, rash, limited kidney and liver functions, and both internal and external bleeding. Specialized laboratory tests on blood specimens (which are not commercially available) detect specific antigens or antibodies and/or isolate the virus. These tests present an extreme biohazard and are only conducted under maximum containment conditions.

No specific treatment or vaccine exists for Ebola hemorrhagic fever. Severe cases require intensive supportive care, as patients are frequently dehydrated and in need of intravenous fluids. Experimental studies involving the use of hyperimmune sera on animals demonstrated no long-term protection against the disease after interruption of therapy.

Suspected cases should be isolated from other patients and strict barrier nursing techniques practiced. All hospital personnel should be briefed on the nature of the disease and its routes of transmission. Particular emphasis should be placed on ensuring that high-risk procedures such as the placing of intravenous lines and the handling of blood, secretions, catheters, and suction devices are done under barrier nursing conditions. Hospital staff should have individual gowns, gloves, and masks. Gloves and masks must not be reused unless disinfected. Patients who die from the disease should be promptly buried or cremated.

As the primary mode of person-to-person transmission is contact with contaminated blood, secretion, or body fluids, any person who has had close physical contact with patients should be kept under strict surveillance, i.e. body temperature checks twice a day, with immediate hospitalization and strict isolation recommended in case of temperatures above 38.3° C (101° F). Casual contacts should be placed on alert and asked to report any fever. Surveillance of suspected cases should continue for three weeks after the date of their last contact. Hospital personnel who come into close contact with patients or contaminated materials without barrier nursing attire must be considered exposed and put under close supervised surveillance.

The Ebola virus was first identified in a western equatorial province of Sudan and in a nearby region of Zaire in 1976 after significant epidemics in Yamkubu, northern Zaire, and Nzara, southern Sudan. Between June and November 1976 the Ebola virus infected 284 people in Sudan, with 117 deaths. In Zaire there were 318 cases and 280 deaths in September and October. An isolated case occurred in Zaire in 1977 and a second outbreak in Sudan in 1979. In 1989 and 1990, a filovirus, named Ebola-Reston, was isolated in monkeys being held in quarantine in laboratories in Reston, VA, Alice, TX, and Pennsylvania. In the Philippines, Ebola-Reston infections occurred in a quarantine area near Manila for monkeys intended for exportation. A large epidemic occurred in Kikwit, Zaire in 1995 with 315 cases, 244 with fatal outcomes. One human case of Ebola hemorrhagic fever and several cases in chimpanzees were confirmed in Côte d'Ivoire in 1994-95. In Gabon, Ebola hemorrhagic fever was first documented in 1994 and recent outbreaks occurred in February 1996 and July 1996. In all, nearly 1,100 cases with 793 deaths have been documented since the virus was discovered. The natural reservoir of the Ebola virus seems to reside in the rain forests of Africa and Asia but has not yet been identified.

Different hypotheses have been developed to try to uncover the cycle of Ebola. Initially, rodents were suspected, as is the case with Lassa Fever whose reservoir is a wild rodent (*Mastomys*). Another hypothesis is that a plant virus may have caused the infection of vertebrates. Laboratory observation has shown that bats experimentally infected with Ebola do not die and this has raised speculation that these mammals may play a role in maintaining the virus in the tropical forest.

Melioidosis

Melioidosis is an infectious disease of humans and animals caused by *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*), a gram-negative bacillus. It is especially prevalent in Southeast Asia but has been described in many countries around the world. The disease has a variable and inconstant clinical spectrum. A biological warfare attack with this organism would most likely be by the aerosol route.

Infection by inoculation results in a subcutaneous nodule with acute lymphangitis and regional lymphadenitis, generally with fever. Pneumonia may occur after inhalation or hematogenous dissemination of infection. It may vary in intensity from mild to fulminant, usually involves the upper lobes, and often results in cavitation. Pleural effusions are uncommon. An acute fulminant septicemia may occur characterized by rapid appearance of hypotension and shock. A chronic suppurative form may involve virtually any organ in the body.

Antibiotic regimens that have been used successfully include tetracycline, 2-3 g/day; chloramphenicol, 3 g/day; and trimethoprim-sulfamethoxazole, 4 and 20 mg/kg per day. Ceftazidime and piperacillin have enjoyed success in severely ill patients as well. In patients who are toxic, a combination of two antibiotics, given parenterally, is advised.

There are no means of immunization. Vigorous cleansing of abrasions and lacerations may reduce the risk of disease after inoculation of organisms into the skin. There is no information available on the utility of antibiotic prophylaxis after potential exposure, but before the onset of clinical symptoms.

Plague

Plague is a zoonotic disease caused by *Yersinia pestis*. Under natural conditions, humans become infected as a result of contact with rodents, and their fleas. The transmission of the gram-negative coccobacillus is by the bite of the infected flea, *Xenopsylla cheopis*, the oriental rat flea, or *Pulex irritans*, the human flea. Under natural conditions, three syndromes are recognized: bubonic, primary septicemia, or pneumonic. In a biological warfare scenario, the plague bacillus could be delivered via contaminated vectors (fleas) causing the bubonic type or, more likely, via aerosol causing the pneumonic type.

- In bubonic plague, the incubation period ranges from 2 to 10 days. The onset is acute and often fulminant with malaise, high fever, and one or more tender lymph nodes. Inguinal lymphadenitis (bubo) predominates, but cervical and axillary lymph nodes can also be involved. The involved nodes are tender, fluctuant, and necrotic. Bubonic plague may progress spontaneously to the septicemia form with organisms spread to the central nervous system, lungs (producing pneumonic disease), and elsewhere. The mortality is 50 percent in untreated patients with the terminal event being circulatory collapse, hemorrhage, and peripheral thrombosis.
- In primary pneumonic plague, the incubation period is 2 to 3 days. The onset is acute and fulminant with malaise, high fever, chills, headache, myalgia, cough with production of a bloody sputum, and toxemia. The pneumonia progresses rapidly, resulting in dyspnea, stridor, and cyanosis. In untreated patients, the mortality is 100 percent with the terminal event being respiratory failure, circulatory collapse, and a bleeding diathesis.

In cases where bubonic type is suspected, tularemia adenitis, staphylococcal or streptococcal adenitis, meningococemia, enteric gram negative sepsis, and rickettsiosis need to be ruled out. In pneumonic plague, tularemia, anthrax, and staphylococcal enterotoxin B (SEB) agents need to be considered. Continued deterioration without stabilization effectively rules out SEB.

Plague may be spread from person to person by droplets. Strict isolation procedures for all cases are indicated. Streptomycin, tetracycline, and chloramphenicol are highly effective if begun early. Significant reduction in morbidity and mortality is possible if antibiotics are given within the first 24 hours after symptoms of pneumonic plague develop.

A formalin-killed *Y. pestis* vaccine that was produced in the United States and extensively used is no longer manufactured. Efficacy against flea-borne plague was inferred from population studies, but the utility of this vaccine against aerosol challenge was unknown. Immunity was maintained through boosters every 1-2 years. Live-attenuated vaccines are available elsewhere but are highly reactogenic and without proven efficacy against aerosol challenge.

Q Fever

Q fever is a zoonotic disease caused by a rickettsia, *Coxiella burnetii*. The most common animal reservoirs are sheep, cattle and goats. Humans acquire the disease by inhalation of particles contaminated with the organisms. A biological attack would cause disease similar to that occurring naturally.

Following an incubation period of 10-20 days, Q fever generally occurs as a self-limiting febrile illness that includes headache, fatigue, and myalgias and lasts from 2 days to 2 weeks. Pneumonia occurs frequently, usually manifested only by an abnormal chest x-ray. A nonproductive cough and pleuritic chest pain occur in about one-fourth of patients with Q fever pneumonia. Patients usually recover uneventfully.

Q fever usually presents as an undifferentiated febrile illness, or a primary atypical pneumonia, which must be differentiated from pneumonia caused by mycoplasma, Legionnaire's disease, psittacosis or *Chlamydia pneumoniae*. More rapidly progressive forms of pneumonia may look like bacterial pneumonias including tularemia or plague.

Tetracycline (250 mg every 6 hr) or doxycycline (100 mg every 12 hr) for 5-7 days is the treatment of choice. A combination of erythromycin (500 mg every 6 hr) plus rifampin (600 mg per day) is also effective.

Vaccination with a single dose of a killed suspension of *C. burnetii* provides complete protection against naturally occurring Q fever and >90% protection against experimental aerosol exposure in human volunteers. Protection lasts for at least 5 years. However, neither this vaccine nor any other is commercially available in the U.S. Administration of this vaccine in immune individuals may cause severe cutaneous reactions including necrosis at the inoculation site. Newer vaccines are under development. Treatment with tetracycline during the incubation period will delay but not prevent the onset of illness.

Ricin

Ricin is a glycoprotein toxin (66,000 daltons) from the seed of the castor plant. It blocks protein synthesis by altering the rRNA, thus killing the cell. Ricin's significance as a potential biological threat agent relates to its availability worldwide, ease of production, and extreme pulmonary toxicity when inhaled.

Overall, the clinical picture seen depends on the route of exposure. All reported serious or fatal cases of castor bean ingestion have taken approximately the same course: rapid onset of nausea, vomiting, abdominal cramps, and severe diarrhea with vascular collapse; death has occurred on the third day or later. Following inhalation, one might expect nonspecific symptoms of weakness, fever, cough, nausea, and hypothermia followed by hypotension and cardiovascular collapse. High doses by inhalation appear to produce severe enough pulmonary damage to cause death.

In oral intoxication, fever, gastrointestinal involvement, and vascular collapse are prominent, the latter differentiating it from infection with enteric pathogens. With regard to inhalation exposure, nonspecific findings of weakness, fever, vomiting, cough, hypothermia, and hypotension in large numbers of patients might suggest several respiratory pathogens.

Therapy is supportive and should include maintenance of intravascular volume. Standard management for poison ingestion should be employed if intoxication is by the oral route. There is presently no antitoxin available for treatment.

There is currently no prophylaxis approved for human use. Active immunization and passive antibody prophylaxis are under study, as both are effective in protecting animals from death following exposure by intravenous or respiratory routes.

Rift Valley Fever

Rift Valley Fever (RVF) is a viral disease caused by RVF virus. The virus circulates in sub-Saharan Africa as a mosquito-borne agent. Epizootics occur when susceptible domestic animals are infected, and because of the large amount of virus in their serum, amplify infection to biting arthropods. Deaths and abortions among susceptible species such as cattle and sheep constitute a major economic consequence of these epizootics, as well as providing a diagnostic clue and a method of surveillance. Humans become infected through the bite of mosquitoes, contact with infected blood, bodily fluids, or organs, or exposure to virus-laden aerosols or droplets. The human disease appears to be similar whether acquired by aerosol or by mosquito bite. A biological attack, most likely delivered by aerosol, would be expected to elicit the rather specific spectrum of human clinical manifestations and to cause disease in sheep and cattle in the exposed area. If disease occurred in the absence of heavy vector populations or without domestic animals as amplifiers of mosquito infection, a BW attack would also be a likely cause.

The incubation is two to six days and is usually followed by an incapacitating febrile illness of similar duration. The typical physical findings are fever, conjunctival injection, and sometimes abdominal tenderness. A few petechiae or epistaxis may occur. A small proportion of cases (approximately one percent) will progress to a viral hemorrhagic fever syndrome; mortality in this group is roughly 50 percent. A small number of infections will lead to a late encephalitis. After apparent recovery from a typical febrile illness, the patient develops fever, meningeal signs, obtundation, and focal defects. Death is uncommon.

The occurrence of an epidemic with febrile disease, hemorrhagic fever, eye lesions, and encephalitis in different patients would be characteristic of RVF. Demonstration of viral antigen in blood by ELISA is rapid and successful in a high proportion of acute cases of uncomplicated disease or hemorrhagic fever. Other methods of diagnosis include polymerase chain reaction and virus propagation.

In hemorrhagic fever, supportive therapy may be indicated for hepatic and renal failure, as well as replacement of coagulation factors. The virus is sensitive to ribavirin *in vitro* and in rodent models. No studies have been performed in human or the more realistic monkey model to ascertain whether administration to an acutely ill patient would be of benefit.

Avoidance of mosquitoes and contact with fresh blood from dead domestic animals and respiratory protection from small particle aerosols are the mainstays of prevention. An effective inactivated vaccine for humans is not licensed for public use, but is available in limited quantities, particularly to laboratory and veterinary personnel.

Saxitoxin

Saxitoxin is the parent compound of a family of chemically related neurotoxins. In nature they are predominantly produced by marine dinoflagellates, although they have also been identified in association with such diverse organisms as blue-green algae, crabs, and the blue-ringed octopus. Human intoxications are principally due to ingestion of bivalve mollusks which have accumulated dinoflagellates during filter feeding. The resulting intoxication, known as paralytic shellfish poisoning (PSP), is known throughout the world as a severe, life-threatening illness requiring immediate medical intervention. In a BW scenario, the most likely route of delivery is by inhalation or toxic projectile. In addition, saxitoxin could be used in a confined area to contaminate water supplies.

After oral exposure, absorption of toxins from the gastrointestinal tract is rapid. Onset of symptoms typically begins 10-60 minutes after exposure, but may be delayed several hours depending upon the dose and individual idiosyncrasy. Initial symptoms are numbness or tingling of the lips, tongue and fingertips, followed by numbness of the neck and extremities and general muscular incoordination. Nausea and vomiting may be present, but typically occur in a minority of cases. Respiratory distress and flaccid muscular paralysis are the terminal stages and can occur 2-12 hours after intoxication. Death results from respiratory paralysis. Clearance of the toxin is rapid and survivors for 12-24 hours will usually recover. There are no known cases of inhalation exposure to saxitoxin in the medical literature, but data from animal experiments suggest the entire syndrome is compressed and death may occur in minutes.

Routine laboratory evaluation is not particularly helpful. Cardiac conduction defects may develop. Differential diagnosis may require toxin detection. Diagnosis is confirmed by detection of toxin in the food, water, stomach contents or environmental samples.

Management is supportive and standard management of poison ingestion should be employed if intoxication is by the oral route. Toxins are rapidly cleared and excreted in the urine, so diuresis may increase elimination. Incubation and mechanical

respiratory support may be required in severe intoxication. Timely resuscitation would be imperative, albeit very difficult, after inhalation exposure on the battlefield.

No vaccine against saxitoxin exposure has been developed for human use.

Smallpox

Smallpox virus, an orthopoxvirus with a narrow host range confined to humans, was an important cause of morbidity and mortality in the developing world until recent times. Eradication of the natural disease was completed in 1977 and the last human cases (laboratory infections) occurred in 1978. The virus exists today in only 2 laboratory repositories, one in the U.S. and the other in Russia. Appearance of human cases outside the laboratory would signal use of the virus as a biological weapon. Under natural conditions, the virus is transmitted by direct (face-to face) contact with an infected case, by fomites, and occasionally by aerosols. Smallpox virus is highly stable and retains infectivity for long periods outside of the host. A related virus, monkeypox, clinically resembles smallpox and causes sporadic human disease in West and Central Africa.

The incubation period is typically 12 days (range, 10-17 days). The illness begins with a prodrome lasting 2-3 days, with generalized malaise, fever, rigors, headache, and backache. This is followed by defervescence and the appearance of a typical skin eruption characterized by progression over 7-10 days of lesions through successive stages, from macules to papules to vesicles to pustules. The latter finally form crusts and, upon healing, leave depressed depigmented scars. The case fatality rate is approximately 30% in unvaccinated individuals. Permanent joint deformities and blindness may follow recovery. Vaccine immunity may prevent or modify illness.

The eruption of chickenpox (*varicella*) is typically centripetal in distribution (worse on trunk than face and extremities) and characterized by crops of lesions in different stages on development. Chickenpox papules are soft and superficial, compared to the firm, shotty, and deep papules of smallpox. Chickenpox crusts fall off rapidly and usually leave no scar. Monkeypox cannot be easily distinguished from smallpox clinically. Monkeypox occurs only in forested areas of West and Central Africa as a sporadic, zoonotic infection transmitted to humans from wild squirrels. Person-to-person spread is rare and ceases after 1-2 generations. Mortality is 15%. Other diseases that are sometimes confused with smallpox include typhus, secondary syphilis, and malignant measles. Skin samples (scrapings from papules, vesicular fluid, pus, or scabs) may provide a rapid identification of smallpox by direct electron microscopy, agar gel immunoprecipitation, or immunofluorescence.

There is no specific treatment available although some evidence suggests that vaccinia-immune globulin is of some value in treatment if given early in the course of the illness.

Vaccinia virus is a live poxvirus vaccine that induces strong crossprotection against smallpox for at least 5 years and partial protection for 10 years or more. The vaccine is administered by dermal scarification or intradermal jet injection; appearance of a vesicle or pustule within several days is indication of a "take." Vaccinia-immune human globulin at a dose of 0.3 mg/kg body weight provides >70% protection against naturally occurring smallpox if given during the early incubation period. Administration immediately after or within the first 24 hours of exposure would provide the highest level of protection, especially in unvaccinated persons. The antiviral drug, n-methylisatin β -thiosemicarbazone (Marboran[®]) afforded protection in some early trials, but not others, possibly because of noncompliance due to unpleasant gastrointestinal side effects.

Patients with smallpox should be treated by vaccinated personnel using universal precautions. Objects in contact with the patient, including bed linens, clothing, ambulance, etc.; require disinfection by fire, steam, or sodium hypochlorite solution.

Staphylococcal Enterotoxin B (SEB)

Staphylococcal Enterotoxin B (SEB) is one of several exotoxins produced by *Staphylococcus aureus*, causing food poisoning when ingested. A BW attack with aerosol delivery of SEB to the respiratory tract produces a distinct syndrome causing significant morbidity and potential mortality.

The disease begins 1-6 hours after aerosol exposure with the sudden onset of fever, chills, headache, myalgia, and nonproductive cough. In more severe cases, dyspnea and retrosternal chest pain may also be present. Fever, which may reach 103-106° F, can last 2-5 days, but cough may persist 1-4 weeks. In many patients nausea, vomiting, and diarrhea will also occur. In moderately severe laboratory exposures, lost duty time has been <2 weeks, but, based upon animal data, it is anticipated that severe exposures will result in fatalities.

In foodborne SEB intoxication, fever and respiratory involvement are not seen, and gastrointestinal symptoms are prominent. The nonspecific symptoms of fever, nonproductive cough, myalgia, and headache occurring in large numbers of patients in an epidemic setting would suggest any of several infectious respiratory pathogens, particularly influenza, adenovirus, or mycoplasma. In a BW attack with SEB, cases would likely have their onset within a single day, while naturally occurring outbreaks would present over a more prolonged interval.

Treatment is limited to supportive care; humidified oxygen and steroids for pain control. No specific antitoxin for human use is available. There currently is no prophylaxis for SEB intoxication. Experimental immunization has protected monkeys, but no vaccine is presently available for human use.

Trichothecene Mycotoxin

The trichothecene mycotoxins are a diverse group of more than 40 compounds produced by fungi. They strongly inhibit protein synthesis, impair DNA synthesis, alter cell membrane structure and function, and inhibit mitochondrial respiration. Secondary metabolites of fungi, such as T-2 toxin and others, produce toxic reactions called mycotoxicoses upon inhalation or consumption of contaminated food products by humans or animals. Naturally occurring trichothecenes have been identified in agricultural products and have been implicated in the animal disease moldy corn toxicosis, or poisoning.

There are no well-documented cases of clinical exposure of humans to trichothecenes. However, strong circumstantial evidence has associated these toxins with alimentary toxic aleukia (ATA), the fatal epidemic seen in Russia during World War II, and with alleged BW incidents ("yellow rain") in Cambodia, Laos, and Afghanistan.

Consumption of these mycotoxins results in weight loss, vomiting, skin inflammation, bloody diarrhea, diffuse hemorrhage, and possibly death. The onset of illness following acute exposure to T-2 (intravenously or through inhalation) occurs in hours, resulting in the rapid onset of circulatory shock characterized by reduced cardiac output, arterial hypotension, lactic acidosis and death within 12 hours.

Clinical signs and symptoms of ATA were hemorrhage, leukopenia, ulcerative pharyngitis, and depletion of bone marrow. The purported use of T-2 as a BW agent resulted in acute exposure via inhalation and/or dermal routes, as well as oral exposure upon consumption of contaminated food products and water. Alleged victims reported painful skin lesions, lightheadedness, dyspnea, and a rapid onset of hemorrhage, incapacitation and death. Survivors developed a radiation-like sickness including fever, nausea, vomiting, diarrhea, leukopenia, bleeding, and sepsis.

Specific diagnostic modalities are limited to reference laboratories. Because of their long "half-life" the toxic metabolites can be detected as late as 28 days after exposure.

General supportive measures are used to alleviate acute T-2 toxicoses. Prompt soap and water wash within 5-60 min of exposure significantly reduces the development of the localized destructive, cutaneous effects of the toxin. Oral exposure management should include standard therapy for poison ingestion.

Ascorbic acid (400-1200 mg/kg, inter-peritoneal (ip)) works to decrease lethality in animal studies, but has not been tested in humans. While not yet available for humans, administration of large doses of monoclonal antibodies directed against T-2 and other metabolites have shown prophylactic and therapeutic efficacy in animal models.

Tularemia

Tularemia is a zoonotic disease caused by *Francisella tularensis*, a gram-negative bacillus. Humans acquire the disease under natural conditions through inoculation of skin or mucous membranes with blood or tissue fluids of infected animals, or bites of infected deerflies, mosquitoes, or ticks. A BW attack with *F. tularensis* delivered by aerosol would primarily cause typhoidal tularemia, a syndrome expected to have a case fatality rate which may be higher than the 5-10% seen when disease is acquired naturally.

A variety of clinical forms of tularemia are seen, depending upon the route of inoculation and virulence of the strain. In humans, as few as 10-50 organisms will cause disease if inhaled or injected intradermally, whereas 10^8 organisms are required with oral challenge. Under natural conditions, ulceroglandular tularemia generally occurs about 3 days after intradermal inoculation (range 2-10 days), and manifests as regional lymphadenopathy, fever, chills, headache, and malaise, with or without a cutaneous ulcer. Gastrointestinal tularemia occurs after drinking contaminated ground water, and is characterized by abdominal pain, nausea, vomiting, and diarrhea. Bacteremia may be common after primary intradermal, respiratory, or gastrointestinal infection with *F. tularensis* and could result in septicemia or "typhoidal" tularemia. The typhoidal form also may occur as a primary condition in 5-15% of naturally-occurring cases; clinical features include fever, prostration, and weight loss, but without adenopathy. Diagnosis of primary typhoidal tularemia is difficult, as signs and symptoms are nonspecific and there frequently is no suggestive exposure history. Pneumonic tularemia is a severe atypical pneumonia that may be fulminant, and can be primary or secondary. Primary pneumonia may follow direct inhalation of infectious aerosols, or may result from aspiration of organisms in cases of pharyngeal tularemia. Pneumonic tularemia causes fever, headache, malaise, substernal discomfort, and a non-productive cough; radiologic evidence of pneumonia or mediastinal lymphadenopathy may or may not be present. A biological warfare attack with *F. tularensis* would most likely be delivered by aerosol, causing primarily typhoidal tularemia. Many exposed individuals would develop pneumonic tularemia (primary or secondary), but clinical pneumonia may be absent or non-evident. Case fatality rates may be higher than the 5-10% seen when the disease is acquired naturally.

The clinical presentation of tularemia may be severe, yet nonspecific. Differential diagnoses include typhoidal syndromes (e.g., salmonella, rickettsia, malaria) or pneumonic processes (e.g., plague, mycoplasma, SEB). A clue to the diagnosis of tularemia delivered as a BW agent might be a large number of temporally clustered patients presenting with similar systemic illnesses, a proportion of whom will have a nonproductive pneumonia. Identification of organisms by staining ulcer fluids or sputum is generally not helpful. Routine culture is difficult, due to unusual growth requirements and/or overgrowth of commensal bacteria.

Streptomycin (1 gm every 12 hours intramuscularly (IM) for 10-14 days) is the treatment of choice. Gentamicin also is effective (3-5 mg/kg/day parenterally for 10-14 days). Tetracycline and chloramphenicol treatment are effective as well, but are associated with a significant relapse rate. Although laboratory-related infections with this organism are very common, human-to-human spread is unusual and isolation is not required.

A live, attenuated tularemia vaccine is available as an investigational new drug (IND). This vaccine has been administered to more than 5,000 persons without significant adverse reactions and is of proven effectiveness in preventing laboratory-acquired typhoidal tularemia. Its effectiveness against the concentrated bacterial challenge expected in a BW attack is unproven. The use of antibiotics for prophylaxis against tularemia is controversial.

Venezuelan Equine Encephalitis

Eight serologically distinct viruses belonging to the Venezuelan equine encephalitis (VEE) complex have been associated with human disease; major human outbreaks have been associated with subtype 1, variants A, B and C. These agents also cause severe disease in horses, mules, and donkeys (*Equidae*). Natural infections are acquired by the bites of a wide variety of mosquitoes; *Equidae* serve as the viremic hosts and source of mosquito infection. In natural human epidemics, severe and often fatal encephalitis in *Equidae* always precedes that in humans. A BW attack with virus disseminated as an aerosol would cause human disease as a primary event. If *Equidae* were present, disease in these animals would occur simultaneously with human disease. Secondary spread by human-to-human transmission has not been demonstrated. However, a BW attack in a region populated by *Equidae* and appropriate mosquito vectors could initiate an epizootic/epidemic.

Nearly 100% of those infected suffer an overt illness. After an incubation period of 1-5 days, onset of illness is extremely sudden, with generalized malaise, spiking fever, rigors, severe headache, photophobia, myalgia in the legs and lumbosacral area. Nausea, vomiting, cough, sore throat, and diarrhea may follow. This acute phase lasts 24-72 hours. A prolonged period of asthenia and lethargy may follow, with full health and activity regained only after 1-2 weeks. Approximately 4-14% of children and <1% of adults during natural epidemics develop signs of central nervous system infection, with meningismus, convulsions, coma, and paralysis. These neurologic cases are seen almost exclusively in children. The overall case-fatality rate is <1%, but in children with encephalitis, it may reach 20%.

An outbreak of VEE may be difficult to distinguish from influenza on clinical grounds. Clues to the diagnosis are the appearance of a small proportion of neurological cases or disease in *Equidae*, but these might be absent in a BW attack.

There is no specific therapy. Patients who develop encephalitis may require anticonvulsant and intensive supportive care to provide adequate ventilation, maintain fluid and electrolyte balance, and avoid complicating secondary bacterial infections.

An experimental vaccine, designated TC-83 is a live, attenuated cell culture-propagated vaccine which has been used in several thousand persons to prevent laboratory infections. Approximately 10% of vaccines fail to develop detectable neutralizing antibodies, but it is unknown whether they are susceptible to clinical infection if challenged. A second investigational product that has been tested in humans is the C-84 vaccine, prepared by formalin-inactivation of the TC-83 strain. Both vaccines are not licensed for general public use. In experimental animals, alpha-interferon and the interferon-inducer poly-ICLC (lysine-polyadenosine) have proven highly effective for post-exposure prophylaxis of VEE. There are no clinical data on which to assess efficacy in humans.

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