(U) Anthrax

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(U) **Anthrax** is a serious illness caused by *Bacillus anthracis*, an encapsulated, spore-forming, large, gram-positive, aerobic (grows in the presence of air), nonmotile (unable to move independently), rod-shaped bacterium. It is primarily a disease of plant-eating animals (herbivores); cattle, sheep, goats, horses, and swine are the usual hosts. The bacteria grow within the host, and sporulation occurs when the bacteria are exposed to oxygen or adverse growing conditions. Virulent strains of *B. anthracis* produce a protective capsule, composed of poly-D glutamic acid, and two protein exotoxins (called the lethal and the edema toxins).

(U) Definition in the WMD Term Handbook An infectious disease of cattle and sheep caused by the bacterium Bacillus anthracis. The disease can be transmitted to human by infected aerosol inhalation anthrax or the respiratory form presents as a flu-like illness with fever, fatigue, nonproductive cough and chest discomfort all of which last 2 to 3 days. It may then progress to pneumonia with respiratory distress, shock, and meningitis generally leading to death in 24 to 36 hours despite appropriate therapy. Person to person spread does not occur. However the disease can be transmitted through contact of contaminated animal substances such as hair, bones, or hides. Direct contact of infected materials with the skin (cutaneous form) leads to the development of a painless ulcer and swollen lymph nodes. Gastrointestinal anthrax occurs after ingestion of contaminated poorly

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cooked meat or bone meal. The therapeutic agents of choice are ciprofloxacin, doxycycline, and penicillin plus streptomycin. Ciprofloxacin in combination with anthrax vaccine provides effective prophylaxis in case of exposure.[1]

Type

(U) **Bacillus anthracis** is a bacterium.[2] This bacterium is the causative agent of anthrax. This organism has the ability to remain viable for decades in the environment, especially the soil, in the form of spores. Spores are the most highly infectious phase of the bacterial life cycle.[3]

Names

Anthrax is also known as woolsorter's disease, splenic fever, Siberian ulcer, Siberian boil plague, rag-pickers disease, Persian fever, milzbrand, malignant pustule (pustula maligna), malignant carbuncle, and charbon.[2]

Simulants

The behavior of *Bacillus anthracis* can be simulated by *Bacillus subtilis* var. niger (*Bacillus globigii*), and *Bacillus thuringiensis*.[4][5]

History

Anthrax has been traced back to the fifth and sixth plagues of Egypt, around 1500 B.C. The anthrax disease, known during that era as "black bane" or "Murrain," caused serious losses of cattle. In the early 1700s, anthrax first appeared in North America in Louisiana. Cutaneous anthrax later appeared itself among cowboys on cattle ranches in Kentucky in 1824. In 1864, anthrax was linked as a disease caused by microorganisms living in the blood of infected animals.

During the 1930s, extensive research was conducted in Germany, Russia, and Japan

toward the use of anthrax as a biological weapon. During World War II, several countries produced anthrax, yet Japan was the only country to use it as a biological warfare agent (Unit 731). Japan produced mass quantities of anthrax from 1939 until 1945.

Intelligence History Portal

Anthrax has surfaced in the news in the post-World War II era. In support of the Biological Weapons Convention, the United States announced in 1969 that it would destroy all stockpiles of biological agents and weapons, including anthrax. Also, two anthrax epidemics occurred in the 1970s. The first occurred among cattle in Rhodesia (now called Zimbabwe), and the second epidemic occurred in Sverdlovsk. This outbreak resulted from an accidental release of anthrax spores from a production facility known locally as Cantonment 19. In 1980, it was reported that Iranian prisoners of war died while undergoing anthrax testing. In the 1990s, North Korea allegedly tested anthrax and other bioagents. In 1995, it was discovered that Iraq had conducted weapons trials using anthrax as the fill agent.

For more information, see History of Anthrax

Description/Symptoms

B. anthracis bacteria are encapsulated, spore-forming, large, gram-positive, aerobic (grows in the presence of air), nonmotile (unable to move independently), and rod-shaped. It is primarily a disease of plant-eating animals; cattle and sheep are common hosts.

B. anthracis spores are extremely resistant to environmental factors. They can remain viable for several decades under suitable environmental conditions. There are three forms of anthrax: inhalational, gastrointestinal, and cutaneous. The incubation period for anthrax is 1 to 7 days, with most cases occurring within 2 days of exposure. The infection usually lasts from 3 to 5 days. Each form of infection is unique with its own characteristic symptoms.

Inhalational anthrax is the manifestation of the disease likely to be expected in biological warfare. The symptoms of inhalational anthrax may vary. (b)(1) sudden death is common, due to blood poisoning (septicemia) or inflammation of the lymph nodes (lymphadenitis). If the dose is small and the particle size is large (b)(1) there are usually notable symptoms. Early symptoms include malaise (discomfort), fatigue, fever, and an upper respiratory infection. These symptoms are followed by the sudden onset of respiratory distress, which is followed by shock; death occurs within 24 to 36 hours. Death occurs in 95 to 100 percent of untreated cases.

Gastrointestinal anthrax shows symptoms of abdominal distress followed by bloody stools, vomiting, fever, and signs of septicemia. Death occurs in about 50 percent of all cases.

Cutaneous anthrax, also referred to as malignant pustule, malignant carbuncle woolsorter's disease, or rag-picker's disease, develops when exposed abraded skin is infected with the bacteria. First, the skin itches, and within 2 to 5 days a skin ulcer (lesion) forms. Later the lesion turns into a large black scab. Untreated infections can spread to regional lymph nodes, causing blood poisoning. Meningitis can also occur. Approximately 5 to 20 percent of untreated patients develop septicemia and generalized infection. It must be emphasized that sudden unexpected collapse and death are the most characteristic indication of anthrax infections, especially in the inhalation form of the disease.

B. anthracis is found in soil (particularly dry soil) as a resistant spore that may persist for years under suitable environmental conditions. Spores vegetate in the soil when the pH and temperature conditions are favorable. The bacteria are found most commonly in areas with somewhat neutral soil (pH 6 to 8.5) and during periods of both drought and flooding. Flooding allows the bacteria to accumulate at the ground surface in low-lying areas. Subsequent drought affords conditions for exposure of the spores. The areas of the world where anthrax is endemic (prevalent) in animals are the Middle East, Africa, and Central and South America. The spores are very resistant to heat, disinfectants, sunlight, and other environmental factors. When the spores are inhaled, they convert to the vegetative form, establish an infection, and, as they multiply in the host, produce highly lethal toxins. There are three primary classifications of infection by B. anthracis: cutaneous,

gastrointestinal, inhalational. The incubation period of anthrax is 1 to 7 days, with most cases occurring within 2 days of exposure. The infection usually lasts from 3 to 5 days. Each form of infection is unique with its own characteristic symptoms.[6][7][8]

Inhalational anthrax

When the dose of inhalational anthrax is large and the particle size is small (b)(1)
death is expected once symptoms occur. Because the average anthrax spore is 2.6 microns, it is small enough to (b)(1)
pass through the upper respiratory tract and penetrate the lungs. Anthrax spores are transported by host cellular immune defense processes. This causes septicemia (blood poisoning) or hemorrhagic inflammation of the lymph nodes (lymphadenitis). This is the type of anthrax expected to occur in biological warfare. After a short period of moderate general disability and the rapid onset of septicemia death occurs.[9]

When the patient is infected with a small dose and a large particle size most of the particles settle in (b)(1)
the upper respiratory tract, such as the nasal passages and upper lungs, and do not reach the alveoli. The incubation period is 1 to 6 days. These patients have a prolonged course of prominent bronchial symptoms, which occur in two stages because the particles clump together and cannot pass easily into the bloodstream. In the early stage of large-particle, low-dose inhalation anthrax, the symptoms are mild, nonspecific, and characterized by insidious onset. Symptoms include mild fever, discomfort (malaise), fatigue, muscle pain (myalgia), a nonproductive cough, and frequently a sensation of precordial oppression (a feeling of heaviness in the chest). This initial stage typically lasts for several days. The patient's clinical condition may improve slightly toward the end of this stage. The second stage of inhalational anthrax is acute toxemia. It advances very rapidly and is associated with a massive invasion of the organism throughout the body. It develops with acute difficult breathing (dyspnea) and bluish skin coloring (cyanosis). The patient appears moribund (unable to move), with accelerated pulse and respiration. The body temperature, although usually elevated to 102°F or more, may suddenly become subnormal because of shock. Harsh breathing (stridor) is common, and chest examination discloses crepitant rales (abnormal crackling sound) associated with fluid in the lungs. The average duration of this acute stage is less than 24 hours, terminating in death. Consciousness is usually maintained until death, except in the case of meningitis (which occurs in 50 percent of the cases), when disorientation and coma occur.[10][11]

Gastrointestinal anthrax

The least common, naturally occurring form of anthrax is gastrointestinal anthrax. It may develop secondary to a primary lesion in the region of the mouth, or it may result from the ingestion of large numbers of spores in uncooked or undercooked infected food (primarily meat). The incubation period is from 2-5 days. In the early stages of gastrointestinal anthrax, the symptoms are mild and nonspecific. This early stage is followed by a very rapid onset of the advanced disease, which is associated with a massive invasion of the organism throughout the body. Symptoms of acute gastrointestinal anthrax are severe protracted vomiting, fever, signs of blood poisoning, and bloody diarrhea. The latter is relatively uncommon because intestinal obstructions rapidly develop (adynamic ileus) with symptoms of fulminating, acute, generalized inflammation of the abdominal cavity membranes (peritonitis). No significant involvement of the membrane that lines the abdominal cavity and contains the organs of the abdominal cavity (peritoneum) actually occurs, although a characteristic hemorrhagic inflammation of the lymph nodes (lymphadenitis) in the peritoneal fold encircling the small intestines (mesentery) is invariably present.[10][11]

Cutaneous anthrax

In cutaneous anthrax, also known as woolsorter's disease or rag-picker's disease, invasion occurs through either abraded skin or through small breaks in the layers of cells forming the epidermis of the skin (epithelium). The incubation period is from 1 to 5 days. The signs start with irritated itching where the skin was exposed to the agent. Within a few hours, the affected area appears as a small, red discolored spot or patch on the skin (macule). It progresses over a period of several hours to a firm, red elevated area of skin that is solid and circumscribed (papule). It proceeds to enlarge into a skin ulcer (vesicle) with surrounding fluid buildup (edema). The vesicle formation may be single or multiple with rings of satellite vesicles surrounding the central lesion. The known symptoms for the early stages of cutaneous anthrax are discomfort (malaise), fever, headache, and general exhaustion (prostration). In 2 to 3 days, the vesicle becomes hemorrhagic and is surrounded by a deep red zone of hardened tissue (induration and erythema). The amount of local fluid build-up (edema) is variable and

depends to a certain extent on the location of the lesion. In areas such as the skin of the eyelids, face, and neck, it can be massive and present a serious complication. Under most circumstances, a zone of doughy edema surrounds the primary lesion. During the active phase of the disease, most patients have significant fever, reaching maximum levels of 101°F. By the sixth day, it manifests as a black scab, and fluid buildup subsides. At this point, the lesion and surrounding skin dies (becomes necrotic). The wound is not painful, but the regional lymph nodes are tender. By this stage, blood poisoning (septicemia) and meningitis can occur. The black scab can usually be removed after 10 to 14 days. The severity of the symptoms depends on the stage of the disease and the dose (the number of organisms involved in the initial infection). Approximately 5 to 20 percent of untreated patients will develop septicemia and generalized infection. More than 95 percent of anthrax cases are cutaneous.[10][12][11][13]

Mortality rates

The mortality rate for inhalation anthrax is 95 to 100 percent in untreated cases, in intestinal anthrax, ~50 percent and in cutaneous, ~20 to 30 percent in untreated cases. Mortality rates for oropharyngeal anthrax can be as high as 50 percent.[10][14]

Animal Studies

In studies using laboratory grown-spores and exposure of monkeys to aerosols generated under controlled conditions, the median lethal dose (LD 50) values were established. (b)(1)

How this data relates to humans is yet to be determined.[15]

The symptoms for herbivores (cattle, sheep, swine, and goats) can be broken down into three forms: peracute (violent acute symptoms), acute (rapid onset, severe symptoms, and a short course), and localized (restricted to a region). Peracute forms are seen in cattle, sheep, and goats. These animals suffer from cerebral apoplexy (severe hemorrhage) and die, frequently without showing any previous evidence of illness. In cattle, intermandibular swelling extending into the jugular furrow can be noted. Severe difficult breathing (dyspnea), with head held low and outstretched, is common. Tremors and rapid progression to lying down (recumbency) can occur. The acute form is most common in all species, except swine. The classic symptoms of this type are fever, excitement, depression, stupor, spasms, respiratory or cardiac distress, convulsions, and bloody discharges. In the localized form, swelling and circumscribed inflammation of the skin (carbuncles) in various parts of the body occurs. In swine, anthrax usually is localized in the cervical lymph nodes, where it causes swelling and hemorrhage. This form may progress to the acute stage or subside with recovery. In swine, death may occur from suffocation.[16][17][18]

Diagnostics

Early diagnosis of anthrax is essential to prevent fatalities. A correct diagnosis is not easily accomplished, because the initial symptoms of the disease are not unique to anthrax. The most critical aspect in making a diagnosis of any form of anthrax is a strong suspicion of possible natural, occupational, or battlefield exposure.

Most of the technical methods for confirming a diagnosis of anthrax are useful only in a retrospective analysis, because the patient often dies before conclusive results can be obtained. Available methods include DNA matching, visual examination of smears and cultures, biochemical tests, immunoassays (detection of antibodies to specific antigens), and animal inoculation and skin tests. Each method depends on identification of the disease-causing agent (pathogen) by matching characteristics of a sample to a known pattern. The DNA-matching technique has the greatest potential to confirm a diagnosis of anthrax before the patient dies, because the method is faster (and more specific) than other technical approaches.

Obstacles to Diagnosis

Medical diagnosis is the process of applying scientific methods to establish the cause and nature of a person's illness. The diagnosis is based on a health care practitioner's evaluation of the patient's subjective symptoms, the physical findings, and

the results of various laboratory tests, together with other appropriate diagnostic procedures. Early diagnosis of anthrax is essential to prevent fatalities. Making a correct diagnosis is not easily accomplished because the initial symptoms of the disease are not unique to anthrax. In order to determine the disease that is causing the symptoms, health practitioners often begin by making a differential diagnosis that considers a number of diseases characterized by the patient's symptoms. For example, the differential diagnosis of a respiratory (in the lungs) anthrax epidemic while still in the early stages of nonspecific symptoms could be impeded by its similarities to a wide variety of viral, bacterial, and fungal infectious diseases. The differential diagnosis for cutaneous (on the skin) anthrax could include tularemia, staphylococcal or streptococcal disease, and orf (a viral disease of sheep and goats, transmissible to humans).[19] [20][21][13][15][14]

Diagnosis Based on Exposure History and Signs of Disease

The most critical aspect in making a diagnosis of any form of anthrax is a strong suspicion of possible natural, occupational or battlefield exposure. For cutaneous and naturally occurring respiratory anthrax, exposure most frequently arises from proximity to infected animals or contaminated animal products. Under battlefield conditions, respiratory anthrax results from exposure to a *Bacillus anthracis*-containing aerosol (i.e., weaponized anthrax). Gastrointestinal anthrax is exceedingly difficult to diagnose because of the rarity of the disease. Diagnosis of this form of anthrax is usually based on evidence of an outbreak due to ingestion of contaminated meat.[14]

Once the nature of the exposure is verified, physical signs of disease can be investigated to determine the form of anthrax present. Cutaneous anthrax should be considered if the patient has a painless but itchy, raised reddened area of skin, often with surrounding swelling due to fluid buildup in the tissues (edema), that develops into a black scar. The scar, together with extensive edema, is strongly indicative of cutaneous anthrax.[14]

If respiratory anthrax is suspected, X-rays should be performed to detect evidence of the widening of the mass of organs and tissues separating the lungs (mediastinum). The widening is due to inflammation and fluid buildup, including blood, in the mediastinum. The presence of meningitis, chest wall edema, and fluid buildup in the tissues surrounding the lungs also points to respiratory anthrax.[14][21][13]

Technical Methods to Confirm Diagnosis

Most of the technical methods for confirming a diagnosis of anthrax are useful only in a retrospective analysis, because the patient usually is dead or dying before conclusive results can be obtained. (Between 20 and 100 percent of all untreated forms of anthrax will result in fatalities.) Available methods include DNA matching, visual examination of smears and cultures, biochemical tests, immunoassays (detection of antibodies to specific antigens), and animal inoculation and skin tests. Each method depends on identifying the disease-causing agent (pathogen) by matching characteristics of a sample to a known pattern.[22][14][23]

The DNA-matching technique has the greatest potential of confirming a diagnosis of anthrax before the patient dies, because the method is faster than other technical approaches. Matching DNA sequences is a relatively new approach made possible with the development of recombinant DNA techniques. DNA sequences are matched using the polymerase chain reaction (PCR) blood test kits. PCR utilizes DNA probes to identify bacteria present in trace quantities in the blood. For anthrax, the DNA probes would be fragments of *Bacillus anthracis* (*B. anthracis*) DNA marked with a radioactive isotope or fluorescent dye. These labeled DNA fragments would pair up with DNA in a sample, if the sample contains DNA from the same microorganism. The sensitivity of the test varies according to the length of the DNA fragment used for identification. The longer the fragment, the more specific the identification of the bacteria; a long fragment might include 1,000 DNA base pairs.[23]

Traditional visual examination methods focus on the shape, color, and growth characteristics of bacteria found in samples of infected blood, tissue, or body fluids. *B. anthracis* is a straight rod, 5 to 10 microns long, which does not move spontaneously (nonmotile), grows in the presence of oxygen (aerobic), and produces spores. The microorganism is gram-positive, which means that stained cells retain a bluish color after washing with organic solvents. Only those strains of *B. anthracis* that have a protective outer coating or shell (capsule) and produce toxins are virulent (poisonous and infectious). When grown in the laboratory, the organisms form long, curved chains and within 24 hours can produce large, raised, opaque, grayish-white colonies with an irregular border. This irregularity will occasionally give individual colonies a

comet tail appearance, which is quite characteristic.[10]

The identification of bacterial microorganisms can be done by visual examination of smears (initial samples of infected matter spread on a surface such as a glass slide) and cultures (large colonies of bacteria grown in the laboratory from an initial sample). Smears require microscopic examination to view individual or small groups of microorganisms. Staining techniques have been developed to aid in locating the bacteria. Smears are stained by Gram's method for determining shape and staining properties and by the methods of McFadyean for demonstrating capsules. For blood smears, a Giemsa stain is also satisfactory in demonstrating the presence of capsules.[10]

For cutaneous anthrax, samples are taken from the site of infection. Pathogenic strains of *B. anthracis* can usually be located in the fluids found in cutaneous wounds, or in material scraped from the underside of a crust or scab of a skin wound. When examined in smears from the blood or tissues, the organisms usually are found singly or in pairs with well-defined capsules. The typical culturing method suspends samples in small quantities of saline solution that are inoculated directly onto agar plates (a common solid culture medium using extracts from seaweed) or in broth (a liquid culture medium based on meat extracts).[10]

For respiratory anthrax, samples are taken from blood, sputum (material expelled by coughing), and body fluids drawn from the lungs, spinal cord, or swollen lymph nodes. *B. anthracis* is readily detectable by blood culture with routine media. Occasionally, bacilli may be identified in the centrifuged sediment of blood treated with 3 percent acetic acid solution and stained with Wright's stain. Smears and cultures of pleural fluid (from the membrane surrounding the lungs) and cerebrospinal fluid (from the brain and spinal cord) may also be positive for *B. anthracis*. Impression smears of mediastinal lymph nodes (found near the lungs) and spleen from fatal cases are positive.[21][13][24][25][26]

For respiratory and intestinal anthrax, samples including specimens of vomitus and feces would most likely be contaminated with other bacteria, making them unsuitable for examination or culturing. In these cases, inoculating animals can confirm a diagnosis of anthrax. The best method of inoculating animals, preferably white mice or guinea pigs, with this material is by simply scratching the skin. The anthrax bacillus can gain entrance through the abraded skin more easily than other organisms that may be present. Prior to culturing or incubating such contaminated material, it may be heated to 65°C for 60 minutes, or to 80°C for 30 minutes. Another method is to suspend 0.1 ml of the specimen in 10 mL of 1 or 2 percent phenol for one hour at room temperature. Both of these techniques take advantage of the extraordinary stability of anthrax spores.[27]

Animals also may be inoculated by injecting under the skin in the thigh uncontaminated initial or cultured samples suspended in a small quantity of saline. After inoculation (using either contaminated or uncontaminated control samples), if *B. anthracis* is present, death occurs in 36 to 72 hours with characteristic features of anthrax. Such features include edema at the inoculation site; dark colored, uncoagulated blood; an enlarged, dark, easily broken spleen; and a congested, mahogany-colored liver. The bacteria can be recovered readily from the blood of these animals.[28]

In addition to examination of smears and cultures, various biochemical reactions with sugars can also be used to help identify the organism.(7) The toxic byproducts of the bacteria are often present in sufficient amounts to permit anthrax toxin detection in blood by immunoassay (analysis of antibody reaction to antigens), which can be performed in field laboratories. (4)(5) If laboratory facilities are not available and specimens must be shipped, a sterile cotton swab can be soaked in blood and air dried to promote sporulation. This can then be transported in a suitable sterile container to the nearest laboratory.[29]

A positive skin test to anthraxin (undefined antigen derived from the chemical breakdown of the bacillus and that was developed and evaluated in the former Soviet Union) has also been reported to be of value in the retrospective diagnosis of anthrax. Western countries have limited experience with this test.[30]

Diagnostic methods for Infected Animals

Several laboratory methods for verifying anthrax in ill or dead animals may be employed, including smears, cultures, and animal incubation. Direct microscopic examination of suspected material, when stained satisfactorily, will reveal gram-positive bacilli that are 1 to 1.5 microns in diameter and 5 to 8 microns long. In blood smears, most of the bacilli will be single cells, but short chains may exist if the animal has been dead for a few hours. Inoculating tryptose soy agar plates with infected blood will show medusa-headed colonies in 12 to 24 hours. Postmortem diagnosis can be conducted on

animals by collecting blood from any peripheral blood vessel. A blood smear should be prepared with gram or Giemsa stain, and, if anthrax is the cause of death, large numbers of single to short-chained gram-positive, square-ended bacilli will likely be present.[31][32]

Incubating inoculated guinea pigs or mice is extremely valuable. Ninety-five percent of the deaths resulting from animal inoculation will occur on days 2, 3, and 4; the presence of B. anthracis should then be verified by smears taken from the inoculated animals. Tissues may also be cultured for confirmation if an autopsy has been done; however, opening the carcass, if anthrax is a likely diagnosis, is not advisable. Precautions against personal exposure must be taken.[33][34]

Properties/Persistence

B. anthracis is a gram-positive (stained cells remain bluish even when washed with organic solvents), encapsulated, nonmotile (unable to move independently), aerobic (grows in the presence of air) bacterium. Its colonies are large, flat, opaque, raised, and irregular. It produces rather large and stable spores. The spores are resistant to sunlight, heat, and disinfectants. The optimal temperature for growth is 36 °C.

B. anthracis possesses three known virulence factors: an antiphagocytic capsule and two protein exotoxins, called the lethal and the edema toxins. These toxins enable the bacteria to resist host defenses and invade host tissues via the bloodstream.

The survival period for the spores released in aerosol form is dependent upon release height, weather conditions, speed at which the spores are released (deposition velocity), and height of temperature inversion. Spores in soil proves that spores are more resistant to the sun's rays and other environmental elements when protected by loosely packed sand or dirt than unprotected spores.

Ultraviolet (UV) light affects *B. anthracis* differently depending on whether the bacterium is in the vegetative state or spore form. More than 99 percent of vegetative cells die after 20 seconds of exposure to UV rays; however, it takes 25 minutes of UV exposure to kill the same amount of spores. Because *B. anthracis* spores are stable in the environment, this bacterium is often considered the quintessential biological agent.

Properties of Anthrax

Bacillus anthracis is a gram-positive bacterium. The encapsulated cells appear in long, bamboo-shaped chains with square or concave ends. The structure of the capsule is a high-molecular- weight polypeptide composed of poly-D-glutamic acid. It is nonmotile (unable to move independently), and aerobic (grows in the presence of air). Its cells are large (5-10 microns long and 1-1.2 microns wide), and colonies are flat, opaque, raised, and irregular, with a curled margin. *B. anthracis* grows on ordinary blood agar within 18-24 hours. It produces spores in the center of the bacilli when in a medium or in the environment rather than living tissues. These spores are ovoid, subterminal (band of colors on the end), stable, and do not cause any significant swelling of the cells. The spores are also resistant to sunlight, heat, and disinfectants. The optimal temperature for growth is 36 °C.[35]

B. anthracis has three known virulence factors: an antiphagocytic capsule and two protein exotoxins (called the lethal and edema toxins). The capsule is composed of a polymer of poly-D-glutamic acid, a compound that confers resistance to phagocytosis. It may also contribute to the resistance of anthrax to lysis by serum cationic proteins. The genes encoding the two protein exotoxins are located on a 60-kb plasmid. When bicarbonate, carbon dioxide and temperatures are at increased levels, such as is found in the infected host, transcription of the genes for synthesis of the two toxins, as well as for the capsule, are also increased. [36]

Like many bacterial and plant toxins, the anthrax toxins possess two protein components: a cell-binding, or B, domain and an active, or A, domain that has the toxic and, usually, the enzymatic activity. The two toxins share the B protein, called protective antigen [molecular weight (MW) 83,000). The lethal toxin (lethal in experimental animals) is composed of the protective antigen combined with the A protein, which is known as lethal factor (MW 90,000). The edema toxin, consisting of the same protective antigen together with a third protein, edema factor (MW 89,000), causes edema when injected into the skin of experimental animals. The three toxin proteins have no biological activity alone.[37]

Persistence of Anthrax

The persistence of spores that have been dispersed in aerosol is dependent upon the release height, weather conditions, speed at which the spores are released (deposition velocity), and height of temperature inversion. The persistence of spores in soil was tested [redacted] The results showed that spores partially protected from the sun's rays and other environmental elements by sand or dirt persist longer than unprotected spores. Also, the spores persist longer in loose soil than in packed soil. The spores can survive in the soil for decades and can become airborne again if the surface is disturbed; however, the inhalation hazard is reduced due to the large particle size.[38] (b)(1)

Effect of Ultraviolet Radiation

B. anthracis is affected differently if it is in the spore form or the vegetative cell form. More than 99 percent of the vegetative cells used in testing were killed within 20 seconds of exposure to UV wavelengths, while 25 minutes of UV exposure were needed to kill the same amount of spores. In addition to being less sensitive to UV radiation, spores of *B. anthracis* are much more resistant to drying and heat than vegetative cells. Because *B. anthracis* spores are stable in the environment, anthrax is frequently considered the quintessential biological agent.[39]

Mechanism of Exposure/Effects

B. anthracis begins its cycle of transmission in neutral or alkaline calcerous (chalky) soils that serve as an incubator for the spores. Spores germinate to their vegetative form and multiply to infectious levels when favorable soil, moisture, temperature, and nutrient conditions occur. The bacteria are found most commonly in areas that have periods of drought and flooding. Flooding allows the bacteria to accumulate at the ground surface in low-lying areas. Subsequent drought affords conditions for exposure of the spores. Epidemics tend to occur after heavy rainfall and flooding. Farm animals, especially cows and sheep, become infected when feeding in these areas. People who tend to these animals or come into contact with animal products are at high risk of acquiring anthrax. This is the natural way that anthrax is passed on to humans. Normally, human anthrax is found in agricultural regions of the world.

Anthrax can cause infection in the human body through a number of routes. When it is contracted through abrasions on the skin, it results in cutaneous anthrax. When it is contracted by the ingestion of contaminated meat, it results in intestinal anthrax. When it is contracted by the inhalation of spores, it results in pulmonary, or inhalational, anthrax. The number of particles that enter the bloodstream depends upon the particle size. Very few particles greater than 5 microns in diameter penetrate past the nasal passages or the upper pharynx.

Mode of Transmission

Flies and other biting insects may transmit the disease; however, this is not the standard mode of transmission. Farm animals may become infected by consuming contaminated feed such as bone meal, but they usually contract the disease by grazing on contaminated vegetation. People who tend to these animals or come into contact with animal products are at high risk of acquiring the disease. Dried or processed skins and hides of infected animals may carry spores for years. Human anthrax is normally found in agricultural regions of the world such as Southern Europe, Africa, Australia, Asia, North America, and South America, where animals are frequently infected with anthrax.[40][41][42][43]

Route of Infection

Bacillus anthracis can enter the human body through a number of routes, including abraded skin, contaminated food, and inhaled spores. Each form of the disease is manifested differently according to its route of entry. Cutaneous (skin) anthrax is the most common, naturally occurring form and accounts for nearly 90 percent of all cases. People who work with animal products originating from anthrax-endemic areas are at risk of contracting the disease. Infection occurs from contact with tissues of an animal or from the open wounds of an infected individual. Intestinal anthrax results from ingesting contaminated meat. In industrialized nations, intestinal anthrax is less common than the cutaneous or pulmonary form of the disease; however, intestinal anthrax is much more common than pulmonary anthrax worldwide. The low prevalence of

intestinal anthrax in industrialized nations is presumably due to the stringent laws concerning animals allowed into the food chain. Pulmonary anthrax is a form of the disease that is caused by inhaling spores from contaminated dust, wool, or hair, especially when they are handled in a confined space. When inhaled, *B. anthracis* spores germinate in the alveoli. The spores multiply and release three proteins: edema factor, lethal factor, and protective antigen. In specific combinations, these proteins are potent toxins that enable the bacteria to resist host defenses and invade host tissues via the bloodstream. The anthrax bacteria travel to the intestines and lymph nodes, where they can cause serious infection. (b)(1)

Poisoning

The number of particles that enter the lungs depends upon the particle size. The smaller the particle, the farther it will penetrate the respiratory system. Very few particles greater than 5 microns in diameter penetrate past the nasal passages or the upper pharynx. The average size of the particle penetrating to the lungs is probably in the range of 1.0 to 3.5 microns. (b)(1)

Toxicity

A respiratory dose of *Bacillus anthracis* is nearly always toxic, at least to humans. (b)(1) There is no data as to how this relates to humans; therefore, the monkey dose is the best estimate on the number of spores needed for a toxic dose of anthrax.[47][48][49]

Prevention/Treatment

The best prevention against anthrax is vaccination. Bio Port produces the only licensed anthrax vaccine for human use. The recommended schedule for vaccination is 0.5 ml injected under the skin at 0, 2, and 4 weeks, followed by boosters of 0.5 ml at 6, 12, and 18 months. The vaccine induces an immune response in over 95 percent of humans who receive the initial three doses.

A recommended treatment for those who contract any form of anthrax is to combine vaccination with the administration of antibiotics. Animal studies have also demonstrated the effectiveness of supportive therapies, such as administering oxygen and maintaining blood circulation. Treatment should begin as soon as exposure is suspected or anticipated for respiratory (in the lungs) anthrax, which is the form of the disease most likely to be encountered in biological warfare and the most difficult to diagnose. It is not clear if surviving an anthrax infection confers a lasting immunity for humans. (b)(1)

Animals appearing to be infected or having high temperatures should be given high dosages of antibiotics and be vaccinated as soon as possible. Veterinary vaccines provide protective immunity starting 3 to 5 days after vaccination. Unlike vaccines for humans, veterinary vaccines use live spores from the avirulent strains of anthrax.

Prevention

The best prevention against anthrax is vaccination. The currently available licensed vaccine is most effective against cutaneous (on the skin) anthrax, the most common naturally occurring form of the disease. The vaccine is thought to be effective against respiratory anthrax (based on animal studies), the form of the disease most likely to be encountered in biological warfare. Vaccination is recommended for high-risk populations, such as people in direct contact with anthrax-infected animals or animal products (i.e., farmers, factory workers, and laboratory or medical personnel) and soldiers who are at risk of an anthrax attack. Approximately 150,000 service members were vaccinated between 11 January and 28

February 1991 (25 to 30 percent of the total US forces deployed during the Persian Gulf War). On 3 March 1998, Secretary of Defense William Cohen announced that all U.S. military personnel deployed to the Gulf region would be vaccinated against anthrax. Eventually, all active duty and reserve personnel will be vaccinated.[50][51][52]

Vaccines

Bio Ports produces the only licensed human vaccine against anthrax. The purpose of a vaccine is to stimulate an immune response in the body to establish resistance to the disease. With this vaccine, the body's immune response is stimulated by the presence of protective antigen material, a component of the toxins produced by the *Bacillus anthracis* bacteria. The protective antigen vaccine induces an immune response in over 95 percent of humans who receive the initial three doses. The vaccine should be stored at refrigerator temperature (between 2 and 8 °C) and not frozen.[53][54][55][56]

In the US, the protective antigen vaccine, based on research since the mid-1940s, has replaced the weakened live dose type of vaccine developed by Pasteur. As research continues, there is speculation that the current vaccine will probably be replaced by products of recombinant DNA research developed by the US Army Medical Research Institute of Infectious Diseases; however, there are many arguments against recombinant vaccines. In the former USSR, a weakened live dose is still used for human vaccination. Its developers claim it is reasonably well tolerated and affords some protection against cutaneous anthrax in clinical field trials.[57][58][59]

Inoculation Schedule and Resulting Protection

The recommended schedule for vaccination is 0.5 ml injected under the skin at 0, 2, and 4 weeks, followed by boosters of 0.5 ml at 6, 12, and 18 months. Annual boosters are recommended if the potential for exposure continues. Limited human data suggest that completing only the first three doses of the recommended six-dose primary series can provide good protection against both cutaneous and respiratory anthrax. Studies in rhesus monkeys indicate that good protection against respiratory anthrax is achieved after two doses (1 to 16 days apart) for up to two years. It is likely that two doses in humans would be protective as well, but there is too little information to draw firm conclusions. As with all vaccines, the degree of protection depends upon the magnitude of the exposure; a large dose of the infectious agent could overwhelm the vaccine protection.[60][61]

Recent Russian research has suggested that an altered form of some strains of *Bacillus anthracis* (*B. anthracis*) may overcome vaccine protection. Four scientists from a government research center experimented with adding genetic factors to *B. anthracis*. Their results, published in mid-1997, claimed that adding the ability to destroy red blood cells (hemolysis) to *B. anthracis* allowed the altered strains to defeat immunity (in animals). The scientists also claimed that an altered vaccine could easily be created to defeat such altered strains.[62]

Sensitivities and Side Effects

Vaccination is not appropriate for anyone who is sensitive to any of the vaccine components (i.e., formalin, alum, benzthonium chloride) or has a history of clinical anthrax. For those who are vaccinated the likelihood of side effects is low. Up to six percent of recipients will experience mild discomfort, including tenderness, redness, swelling, and itching, at the inoculation site for up to 72 hours. These reactions peak at one to two days and usually disappear within two to three days. Less than one percent will experience more severe reactions in the general area (arm or leg) of the inoculation, potentially limiting the use of the extremity for one to two days. Modest systemic reactions (i.e., muscle pain, malaise, low-grade fever, and headache) are uncommon and usually last for one to two days. Severe systemic reactions (i.e., Anaphylaxis, an extreme allergic reaction, that precludes additional vaccination) are rare. There are no long-term conditions resulting from local or systemic reactions.[63][64]

Treatment

The most effective treatment for all forms of anthrax is to combine vaccination with the immediate (before symptoms appear if possible) administration of antibiotics. The use of antibiotics keeps the patient alive until the patient's body can build immunity to anthrax via vaccination. Studies on rhesus monkeys have shown that the concurrent use of more than one antibiotic has a synergistic effect (interaction of two or more is greater than sum of individual elements), which increases the

patient's chance for survival. This can also help treat the antibotic-resistant strains of anthrax that have been developed.[65][66][67][68]

Animal studies have also demonstrated the effectiveness of supportive therapies, such as administering oxygen and maintaining blood circulation (e.g., by using isoproterenal). Oxygen administration helps to overcome the decrease in blood oxygen levels caused by anthrax toxins. Using a positive-pressure respirator helped anthrax-infected rhesus monkeys survive. Isoproterenol facilitates circulation and oxygenation of the blood by increasing cardiac output and improving blood circulation in the lungs. Rhesus monkeys given isoproterenol survived the effects of anthrax toxin.[69]

Treatment should begin as soon as exposure is suspected or anticipated for respiratory anthrax. If antibiotic therapy is not begun until after symptoms appear, death is a likely result. The antibiotics may be administered orally or intravenously, depending on the form and severity of the disease. The duration of the administration also depends on the form of the disease.[70][71][72]

Under Battlefield Conditions

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If clinical signs of anthrax occur, patients should continue to receive antibiotic treatment.[73][74][75]

In the event of an anthrax attack, inoculations should begin immediately for those soldiers who did not complete an immunization program. A single 0.5- mL dose of vaccine should be given under the skin, followed by two 0.5-mL doses of the vaccine given two weeks apart. Those previously vaccinated with fewer than three doses should receive a single 0.5-mL booster, and vaccination probably is not necessary for those who have received the entire three-dose primary series. If the vaccine is not available, antibiotics should be continued beyond four weeks while the patient is closely observed to confirm that anthrax is not present.[76]

For Respiratory Anthrax

Respiratory anthrax should be treated with large doses of intravenous antibiotics (two million units administered every two hours). Penicillin, ciprofloxacin, and doxycycline are the preferred drugs. Penicillin is probably the best known and most widely used. Ciprofloxacin and doxycycline are recommended to overcome penicillin-resistant organisms. Tetracycline, erythromycin, and chloramphenicol have also been used successfully. Laboratory tests suggest that gentamicin, cefazolin, cephalothin, vancomycin, clindamycin, and imipenem could also be used successfully to treat anthrax. Streptomycin and penicillin administered together have had synergistic effects on respiratory anthrax disease in rhesus monkeys. Such efficacy remains to be demonstrated in humans.[77][78]

For Cutaneous, Intestinal, and Oropharyngeal Anthrax

Cutaneous anthrax that is localized and does not appear to be spreading may be treated with oral penicillin. If the infection begins to spread or systemic symptoms are present, then intravenous therapy with high-dose penicillin (two million units administered every two hours) should be initiated. Treatment should be continued for 7 to 10 days. This therapy, if effective, will reduce systemic symptoms and swelling. The treatment will not change the evolution of injured skin tissue, which will progress through ulceration, sloughing, and scar formation. Intestinal and oropharyngeal (in the mouth) anthrax should be treated with large doses of intravenous penicillin (two million units administered every two hours).[79][80]

Immunity

It is not clear if surviving an anthrax infection confers a lasting immunity for humans. Hodgson reports two cases of cutaneous anthrax that seemed to result in limited immunity. One case occurred in a veterinary surgeon who had an infected forearm from which *Bacillus anthracis* was isolated. This patient had anthrax three years previously that had been bacteriologically verified and that had healed with scar formation. The second attack was accompanied by minor swelling and was readily cleared by two injections of serum. The second case reported by Hodgson was a teak buyer who developed a non-inflamed blister on the back of his neck. A brownish fluid obtained from the blister contained unidentified bacilli. The

teak buyer apparently had anthrax in severe form five months previously, that had been treated with serum and surgical excision. A second attack, six weeks after the first, was less severe and, when treated in the same fashion as the first, quickly subsided.[81]

For Exposed Animals

All exposed animals, whether or not they appear to be infected or have high temperatures, should be isolated and given antibiotics and vaccinations. Infected and exposed animals should be in a separate location from non-exposed animals and given long-acting antibiotics immediately. High dosages of antibiotics (50 mL of long-acting penicillin; 300,000 IU/mL) are recommended. Such antibiotic therapy can stop anthrax intoxication if given early. Infected or exposed cattle should be vaccinated as soon as possible using Thraxol (Miles Laboratories) or Anthrax Spore Vaccine (Colorado Serum). The vaccine provides protective immunity starting 3 to 5 days after vaccination. A booster vaccination should be given according to label directions. Antibiotic therapy can prevent death until the vaccine can provide immunity.[82]

Veterinary vaccines use live spores of the non-disease-producing strains of anthrax. Antianthrax serum of bovine and equine origin is marketed in 100 mL quantities by the Pitman-Moore Company of Indianapolis, Indiana. Preliminary precautions against serum sensitivity must be observed.[83][84]

Alternate Uses

There are no developed alternative uses for *B. anthracis*; however, a vaccine that uses a potent toxin from *B. anthracis* is being studied by researchers at Harvard Medical School. This vaccine could lead to an entirely new class of human vaccines against most viruses, certain bacteria, and parasites. This development is the first successful attempt to engineer a protein-based vaccine that works by using the immune system's killer T cells. T cells respond to infection and generate a specific immunological memory for future protection. Researchers have designed the vaccine by fusing a harmless piece of an anthrax toxin with a piece of the model pathogen to stimulate the T cells. The transporter component of the anthrax toxin is then added to this mixture and injected into mice. Although the initial work appears promising, it is uncertain whether the vaccine can protect against death by anthrax. Steps still need to be taken before the vaccine is tested on humans.

Vaccine

Although there are no alternative uses for *Bacillus anthracis* (*B. anthracis*), researchers at Harvard Medical School are building an experimental vaccine from a toxin of *B. anthracis*. This vaccine is being used to combat a model pathogen and could lead to an entirely new class of human vaccines against most viruses, certain bacteria, and parasites. Also, it may be helpful in developing cancer vaccines and therapies.[85]

Immune system

The work being done at Harvard represents the first successful attempt to engineer a protein-based vaccine that works by using the body's natural immune system killer T cells. T cells respond to infection and generate a specific immunological memory for future protection. Most current protein-based vaccines, such as the one used against tetanus, stimulate B cells. B cells produce antibodies and only detect invading pathogens so long as they are outside of cells. Once the pathogen invades past this point of defense and slips inside cells of the body, T cells must be activated. This requires that the antigens they combat be displayed to them from inside infected cells. Delivering a vaccine into cells is much more complex than simply injecting it into a person's bloodstream.[86]

Inserting the vaccine

Researchers have engineered an intracellular vaccine that moves the proteins across the cell membrane and into the cytoplasm. A technique has been developed to manipulate some of the toxin's components so that they are innocuous but able to transport any protein. Next, the researchers genetically fuse a harmless piece of the anthrax toxin to a piece of their model pathogen required to stimulate T cells but unable to cause full-blown disease. Finally, they mix the pieces with a transporter component of the anthrax toxin and inject it into mice.[87]

Results of the vaccine

Although the initial work appears promising, steps need to be taken before the vaccine is tested on humans. AIDS patients and initial testing of vaccine information removed; could be misleading.[88]

Production/Storage

In order to produce any type of bacterial agent, including *B. anthracis*, seed stocks were carefully selected to provide the desirable characteristics.

Media production was a relatively simple process. *B. anthracis* grew readily on a wide variety of substrates; however, sporulation could vary widely depending on conditions and media used. The media were stored for several days before they were inoculated with *B. anthracis*, ensuring that the culture medium was sterile and not contaminated with other bacteria.

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Materials Indicating *B. anthracis* Culture and Weaponization[4]

B. anthracis culture or seed stock
Lab Glassware and Supplies
Growth Media
Centrifuge
Incubator
pH Meter
Refrigerator
Thallium Acetate[**]
Freeze Dryer
Microscope
Shaker
Fermenter
Milling Equipment
Fluidizer or Silica

A hazard of *B. anthracis* was the containment of the spores after production. If released into the air, the bacteria could enter either the air-handling system or environment where the persistent spores could contaminate people and the surrounding area.

Pilot Scale Production

Interim Report 108

Experimental Operating Procedure for Manufacturing *B anthracis* in Pilot Plant B-

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(C) As a reference for comparative analysis of state biological warfare programs, this document serves as an excellent guidepost. Technologies presented in this tome are dated, but demonstrate how the US was able to arrive at ton production capabilities for **anthrax** BW agent using extant and newly developed or improvised technologies for BW production. The document has been declassified to Confidential.
Del 20:43, 28 November 2007 (UTC)

BW Production Requirements in the US BW Program (C)

(C) Special report 243 provides unique insights into the challenges posed in 1956 by modifications of an existing production line to adapt to a new biological warfare agent, anthrax. For analysts involved in evaluation of production processes for

biological warfare, the document give real-world examples of how the US modified a processes line to make anthrax biological warfare agent.

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) Since this facility was designed specifically for production and processing of vegetative agents, extensive modification are required to prepare the facility for production of N. Major revisions are required in agent concentration, filling, loading, clustering and waste sterilization areas. These revisions include: installation of vessels for collecting and blending concentrated suspensions of agent from the centrifuges; addition of equipment for filling, loading and clustering munitions; and addition of equipment for decontaminating agent in liquid effluent from potentially contaminated areas. Minor revisions are required in all process areas.

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This document is in 2 parts. [|Part 1] [|Part 2] Del 19:27, 16 November 2007 (UTC)

Laboratory requirements

In order to produce liquid cultures of *Bacillus anthracis*, a well-equipped microbiologgy laboratory, including the following laboratory equipment and supplies is required:

- Mettler balance, model PM600
- Beakers, graduated (100 ml and 600 ml)
- Erlenmeyer flasks, polycarbonate with screw cap (500 ml and 1000 ml)
- Magnetic stirrers
- Magnetic stirring bars
- Microscope
- Glass microscope slides
- Autoclave, portable, electric
- Mini-pH meter
- Pipets, disposable, sterile (5 ml and 10 ml)
- Pipet filler
- Shaker, rotating
- Shaker, flask carrier for 15-500 ml flasks
- Digital thermometers
- Medium components
- Tryptic soy broth
- Peptone
- Glucose
- Plasmolyzed yeast
- K 2HPO 43(H 2O)
- KH 2PO 4
- FeSO 47(H 2O)
- MnSO 44(H 2O)
- MgSO 47H 2O
- CaCl 26(H 2O)[89]

Seed selection

In order to produce a bacterial agent, including *B. anthracis*, seed stocks are carefully selected to provide the desirable characteristics (e.g., stability, virulence, uniformity, good growth and yields, and aerosol stability). After testing for purity and culture characteristics, the seeds are maintained at a liquid nitrogen temperature (195.8 °C) until needed.[90]

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Starter cultures

To start the culture production process, seed stocks are reconstituted with saline solution. The seed stocks must be able to withstand a series of at least five serial transfers for build-up to the desired volume. To create the desired volume, the seed cultures are prepared by inoculating 500 ml of media with stock cultures and incubating them at 35 °C. Fifty milliliters of the stock culture are used to inoculate 500 ml of media. In order to achieve maximum growth and induce spore formation, the cultures are aerated by shaking on a rotating shaker. Once the cultures have achieved maximum growth, as measured by a spectrophotometric evaluation of the turbidity (cloudiness) of the cultures, the culture flasks can be stored in a refrigerator until the desired amount of culture has been obtained.[91]

Media production

Culturing *B. anthracis* is a relatively simple process. It grows readily on a wide variety of substrates (material on which an organism lives); however, sporulation can vary widely depending on conditions and media used.

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Distilled water should be used to prepare media. The medium without glucose is prepared and distributed in 20-ml amounts into 500-ml Erlenmeyer flasks. The flasks are stoppered with cotton plugs and then covered with aluminum foil. The flasks are sterilized at 20 pounds pressure for 20 minutes in an electric autoclave or pressure cooker with a pressure gauge. The glucose is sterilized separately in distilled H 2O. The glucose is added to the medium after cooling. The culture medium is stored for several days before inoculation to ensure that the culture medium is sterile and not contaminated with other bacteria.[92][93]

In the media preparation for industrial size cultivation, the dry or wet media ingredients are added to treated water. The sterile water is contained in an agitated stainless steel, jacketed vessel to allow temperature control. Steam, hot, and cold water can be circulated in the jackets to speed dissolution of the ingredients, as well as hold the media for subsequent sterilization. Generally, sterilization of prepared media is performed in another separate, closed, agitated stainless steel vessel while the fermentor and associated piping is being separately sterilized by high-pressure steam. Following sterilization of the media, the media is transferred under aseptic conditions to the fermentator.

Technical report BWL 12

Evaluation of Media for Growth of *Bacillus anthracis* and *Pasturella tularensis*, Apr 1959

(C) This [|document] focuses on the selection and optimization of nutrient media for industrial-scale production of **anthrax** and tularemia biological warfare agents. The detailed information and references in this document are invaluable resources

for technical evaluations of biological warfare capabilities.
Del 21:26, 28 November 2007 (UTC)

Fermentation

An agitated stainless steel vessel can be used to cultivate *B. anthracis*. Temperature, dissolved oxygen, and pH can be controlled in the fermentor. Production of *B. anthracis* can be accomplished in 5,000-gallon, closed, aerated vessels. Associated piping should also be stainless steel, completely welded, and designed for high-pressure steam sterilization. All valves should be Saunders diaphragm types, modified to allow steam to pass through all areas of the system to ensure complete sterilization. Air for the vessels must be passed through filters to ensure purity and reduce contamination of the cultures.

Centrifugation

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Hazards of production and storage

A hazard of *B. anthracis* production is the containment of the spores after production. If released into the air, the bacteria can enter the air-handling system and the environment where the spores can contaminate the surrounding area. At present,

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Detection

Although current technology cannot provide immediate identification of biological agents, detectors can distinguish between some biological warfare (BW) agents and natural organic matter present in the atmosphere in near real time (seconds to minutes). Also, positive identification of an agent is possible within minutes using identifiers. Because detection devices do not provide immediate recognition of BW agents, detection systems must be used in conjunction with other measures such as medical protection (vaccines and other prophylactic measures), intelligence, and physical protection to provide layered primary defenses against a biological attack.

Role of detection

Because *B. anthracis* aerosols have similar compositions and external characteristics and behave as natural, organic matter present in the atmosphere, detection of *B. anthracis* can be difficult.

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One of the greatest benefits of biological detection is the ability to adjust plans and courses of action to the situation at hand. The following could be a typical scenario if a *B. anthracis* attack occurred. First, intelligence conveys information suggesting a possible anthrax attack. Second, personnel employ detection systems to confirm the presence of the agent. Simultaneously, physical and protective measures are implemented to protect the population from the onset of disease until positive identification has been confirmed. A typical physical measure is the protective mask. It is worn to prevent potential

B. anthracis particles from reaching the lungs. Finally, medical personnel assist the exposed population and vaccinate everyone. A primary concern involving a *B. anthracis* attack is that respiratory anthrax has a mortality rate approaching 100 percent unless treatment is begun before signs and symptoms of the disease appear. If anthrax is detected before symptoms appear, the chances of survival are increased because vaccines and other therapeutic measures can be administered. Interim systems of detecting biological agents are being fielded in limited numbers. Without these measures, the first indication of an attack could be the unprotected, ill soldier.[97][98]

Architectural hardware

Since 1992 and Operation Desert Storm, there has been increased interest in the development of biological agent detection systems. Most bio-detection systems currently in use or under development have several functional components. These components include a trigger, a collector, a detector, and an identifier. Since actual biological warfare (BW) attacks vary, the detection equipment used depends upon the situation at hand. Therefore, it is important to understand the specific functions for each piece of equipment. The following paragraphs describe the function of each device, and the tables at the end of this article give examples of the many components available.[99]

Triggers

The trigger's function is to provide an early warning that a change in background air has occurred. Unless a trigger is used before and during an actual airborne release of the anthrax agent, it will not offer a significant response since it can only verify a change in the air. The indicators used to identify a change in the background levels are an increase in the aerosol particle count and an increase in the fluorescence of biological-type aerosols.[99]

Collectors

A collector or concentrator samples the atmosphere and concentrates the airborne particles into a liquid medium for analysis. A collector is beneficial to use when an anthrax attack occurs, because the artificial particles are present in the air. When a collector is used with a trigger, the trigger relays a signal to the collector indicating a change in the background level. As soon as the collector receives the signal, an air sample is collected and airborne particles are concentrated into a liquid medium. This scenario would take place only in a situation where continuous monitoring is occurring.[99]

Detectors

Detectors indicate the presence of biological matter in a collected sample. They are non-specific in their detection method; therefore, they respond to both pathogens (something that causes disease) and non-pathogens. Because detectors are non-specific to the biological substance they detect, specific identifiers must be used to confirm the presence of a BW agent. In a usual scenario, a trigger responds to a change, a sample is collected, a detector then senses biological materials, and an identifier confirms the presence of a specific BW agent, such as anthrax. (see Table 3).

Detectors indicate the presence of biological matter in a collected sample. They are non-specific in their detection method; therefore, they respond to both pathogens (something that causes disease) and non-pathogens. Because detectors are non-specific to the biological substance they detect, specific identifiers must be used to confirm the presence of a BW agent. In a usual scenario, a trigger responds to a change, a sample is collected, a detector then senses biological materials, and an identifier confirms the presence of a specific BW agent, such as anthrax.[99]

Identifiers

The role of identifiers is to analyze a collected liquid sample to determine whether a particular BW agent, such as anthrax, is present in the sample. Identifiers are typically based on antigen-antibody responses. There are three methods of feeding a sample to an identifier. The first method is pumping the sample directly into the identifier from the collector. The second method is using a syringe to inject the sample into the identifier's inlet line. The sample is analyzed for the presence of anthrax. This is usually done in laboratory settings. The final method feeds a sample to an identifier by placing droplets of the collected sample on "Tickets." This mainly requires taking a field sample using a swipe kit.[99]

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Point detectors

One method of biological warfare (BW) agent detection is use of the point detector. An example of a point detector is an air sampler. Air samplers, collect samples of viable particles in multiple stages. (b)(1)
Each stage contains a number of precision-drilled orifices; they are a constant size for each stage. The size of the orifice is based on the particle size desired to be collected in that stage. Orifice sizes decrease with each succeeding impactor stage. Particle-laden air enters the instrument (drawn into the impactor using a blower or pump of some type), and the airborne particles are directed towards the collection surface by the jet orifices. Any particle not collected by that stage follows the stream of air around the edge of the collection surface to the next stage. The collection plate is typically a petri dish with agar (growth media). A selective agar specific to the organism or an all-purpose bacteriological medium can be used. After sampling, the collection plates are removed, covered, and placed in an incubator. After incubation, the number of colonies on each plate is counted using standard bacteriological counting techniques. These samples must be given to another device for anthrax detection (i.e., to identifiers), because air samplers cannot detect agents, they only sample air.[100]

Mobile Labs

Another method of detection is the use of mobile laboratories, such as the (b)(1)
tests environmental air samples by concentrating appropriate aerosol particle sizes in the air, then subjecting the sample to antibody-based detection for selected agents. An improved version is under development to upgrade and expand point detection capabilities.[101][102]

Another example of a mobile laboratory was designed during
that identified potential biological warfare (BW) agents in air (b)(1)
samples. Also, the system used a rapid, whole cell enzyme-linked immunosorbent assay (ELISA) which quickly identified selected bacterial agents. The ELISA immunoassay test depends on the detection of a highly specific reaction (binding) of antigens with their corresponding antibodies (antigen-antibody complex). In an immunoassay-based BW agent identification system, the presence of an agent is detected and identified by relying on the specificity of the antigen-antibody binding agent. In the Persian Gulf, early research concentrated on the identification of *Bacillus anthracis* whole cells, and the resulting assays were fielded in the Persian Gulf. Anthrax could be reliably detected in 5.5 hours. An assay with shortened incubation times was later developed (assay run time of 3.0 to 3.5 hours).[103][104]

Stand-off detectors

The third method of detection is stand-off detectors. Two examples are the , (b)(1)
is in the research and development phase.
Both systems use (b)(1)

Remote detection of biological warfare (BW) agents is best accomplished using
(b)(1)
Both methods of detection will provide early warning, avoid contamination, and point out other detection assets.[106]

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Anthrax as a Biological Warfare Agent

US CBW Program Data

(U/FOUO) This 1972 compilation of data derived from US BW program activity is unique and not available elsewhere. It is a valuable analytical tool, This is one of a set of Volumes created for the purpose of retaining the unique information that was derived from actual weapons research. This Secret document is exempt from declassification.

(U) [|Part One of Volume VII of the Joint CB Technical Data Source Book part 1] [| Part 2]presents the characteristics of the bacterium, *Bacillus Anthracis*, that relate to the possible use of the organism as an agent in biological warfare and to defense against such use. Parameter values are presented for *B. anthracis* that may be used with the general models for predicting weapons effects presented in Volume X. In addition, where adequate information vas available; models and parameter values unique to *B. anthracis* are presented in this volume. These weapons effects estimates may then be used to assess defensive requirements. Del 15:32, 16 August 2007 (UTC)

Weaponization/Dispersal

Anthrax is considered the prototypical biological warfare (BW) agent. Its spore-forming ability makes anthrax well suited for delivery by missiles or bombs. The weapons that were used to carry anthrax were delivery system. These weapons were designed to be loaded into a rocket or missile warhead. (b)(1) (b)(1) Each type of weapon was capable of housing either a liquid or dry agent fill. Anthrax could have been employed as a strategic, tactical, or covert weapon. In its strategic capability, anthrax could have been used for preemptive attacks, but only if a country had the appropriate delivery systems. In its tactical capacity, anthrax could have been used for attacks on reserve formations and service support organizations. In its covert capacity, anthrax could have been used to attack agricultural systems or as an anti-animal weapon. (b)(1)

Weapon Types

Anthrax is considered to be the prototypical biological warfare agent. Its spore-forming ability makes it well suited for delivery by missiles or bombs. Anthrax can be used as a strategic, tactical, and covert weapon. Each type of weapon is described in the following paragraphs.

As a strategic weapon, anthrax could be used for preemptive or initiating attacks, but only if the attacking country has appropriate delivery systems and has taken into consideration the time (three to five days) that the infection takes to cause mortality. (b)(1) to disseminate anthrax, (b)(1) either overtly or covertly. In the strategic role, an anthrax warhead could be used as a wide-area-effect weapon that causes a country's medical system to be overwhelmed and closes airfields/airports or seaports.

As a tactical weapon, anthrax could be used for attacks on reserve formations and service support organizations. (b)(1)

As a covert weapon, anthrax could be used as an anti-animal weapon. It could also be used as an assassination weapon; however, because anthrax is slow acting, it may not be effective. Anthrax could also be used as an antipersonnel weapon (b)(1)

Bomblets

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(b)(1)

Types of Bomblets

(b)(1)

Line Source Weapons

(b)(1)

Other Delivery Systems

A wide range of potential delivery systems was studied in the United States prior to 1969. It was found that these systems examined the release of dry agent as well as wet releases.

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Meteorological Conditions for Release

Meteorological conditions necessitate precise planning of a biological warfare (BW) attack. *Bacillus anthracis* is refractory (resistant) to sunlight. Wind is also an important factor in preplanning a BW attack.

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(b)(1)

When anthrax is used with an (b)(1)

When discussing aerosols, it is important to discuss the level at which primary infection is initiated or the degree of penetration into the trachea-bronchial tree. To a great extent, the size and mass of the inhaled particle determine initiation and penetration. (b)(1)

Very few particles greater than five microns in diameter penetrate past the nasal passages.[119]

Dispersal Efficiency

The dispersal efficiency for anthrax depends on the type of weapon or device used to disseminate the agent and whether the agent is in liquid or dry form. The following chart compares the efficiency of liquid and dry anthrax dissemination from bomblet and line-source weapons. [120]

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The means by which a biological warfare (BW) agent is delivered on target is associated with the munition; frequently, the munition dictates the delivery system. (b)(1)

With the evolution of sophisticated line-source hardware, the agent, munition, and delivery system must be carefully integrated.[121]

Meteorological Conditions for Dispersal

The meteorological conditions required for anthrax dissemination necessitate precise planning of a biological warfare (BW) attack. Most BW attacks, regardless of agent, are likely to occur shortly before daybreak, at sunset, or at night. Wind is an important factor in preplanning a BW attack.

Thus, the effects on the target cannot be predicted. Liquids and dry agents can be disseminated effectively over a wide range of environments--sub-freezing (for dry agent) temperatures, hot tropical conditions, dry-desert conditions, and during moderate rain and snowfall. As a general rule, (b)(1) (b)(1)

Physical Protection/Decontamination

Physical protection from biological hazards requires the use of respiratory protective equipment. One of the most important items of personal protective equipment is the protective mask and associated filters. This type of protection dramatically reduces inhalation exposure to anthrax spores. The protective value of the mask is critically dependent on the fit.

Protective clothing provides a barrier between a soldier and potentially hazardous agents. The various levels of protective clothing are dependent upon the anticipated presence of the agent.

Decontamination is the process by which an agent is removed or the agent concentration is decreased so that it no longer poses a hazard. When anthrax spores contaminate the soil and vegetation via aerosol dispersal, decontamination of those

areas is nearly impossible. Methods of decontamination for bacterial agents include burning the area or spraying it with a mixture of bleach and water. Chemicals used for decontamination of other biological agents may be used to decontaminate areas exposed to anthrax. Absolute proof of soil and vegetation decontamination is difficult, because anthrax is a naturally occurring organism. Protective Mask

Using a protective mask dramatically reduces potential exposure to inhaled *Bacillus anthracis* spores. The protective value of the mask is critically dependent on the fit. If fitted improperly, the protective ratio afforded by the mask has been measured to be as low as 10, which would not provide an effective barrier to B. anthracis spores.[123] (b)(1)

The respirator mask is designed for use with a specific type of filter. Some masks are designed to be worn with two filters.[124]

Aerosol delivery systems for biological warfare agents most commonly generate invisible clouds with particles or droplets of less than ten micrometers. They can remain suspended for extended periods. Particles may adhere to individuals, their clothing, or their masks.[125]

Protective Clothing

There are various levels of protective clothing. The level of clothing required is dependent on the anticipated magnitude of agent exposure. Protective clothing prevents chemical and biological agents from penetrating through layers of clothing and contacting the skin. Permeable protective clothing allows air and moisture to pass through the fabric without hindering the protection capabilities of the garment. The overgarment, undergarment, gloves, and boots are considered protective clothing.[126]

Decontamination Exposure

In a biological attack, anthrax would likely be dispersed as an aerosol, exposing large areas of soil and vegetation to anthrax spores. Decontamination of areas exposed to anthrax is nearly impossible. It takes decades of weathering and human intervention to properly decontaminate an area exposed to high doses of anthrax. In infected livestock, the bacteria might create new reservoirs for the disease, making the affected area impossible to use until the area is totally decontaminated. Animals dying from anthrax often bleed from their body orifices prior to death, thereby contaminating soil or bedding. Animal carcasses infected with anthrax should be incinerated at the site of death.[127][128][129]

Methods of Decontamination

Decontamination methods for all types of bacterial agents include burning the area or spraying the area with a mixture of bleach and water [seven parts Supertropical Bleach (STB) and 93 parts water]. Spraying water or oil on the area helps prevent secondary aerosol exposure, but does not decontaminate the bacteria. Anthrax spores are highly resistant to decontamination.[130]

Chemicals Used for Decontamination

Any commercial hypochlorite (bleach) can be used to produce a decontaminant that will rapidly kill all potential biological threat agents, including *Bacillus anthracis* spores. Chlorine dosages sufficient to kill anthrax spores rapidly would kill other microorganisms even faster. Sodium hypochlorite, formaldehyde, and phenol are also effective sporicidal decontaminants. These chemicals are caustic and corrosive in addition to being toxic and offensive to humans and animals. A new commercial sporicidal product, Exspor, has been found to be less corrosive than hypochlorite bleach, not caustic, and generallyharmless to humans; however, inhalation of the aerosolized vapors during decontamination may result in breathing difficulties due to the acidity of the solution.[131][132]

Chemicals used for the decontamination of other biological agents are also of some use for anthrax exposures. These chemicals include:

- 10 percent sodium hydroxide (caustic soda or lye) or potassium hydroxide (caustic potash) decontaminant combined with water (highly corrosive and toxic)
- Calcium hypochloride: faster acting than STB; only use if STB is not available; can be used in dry or slurry form
- Formalin (formaldehyde): recommended as decontaminant for relatively closed areas
- Peracetic acid: used on equipment and utensils
- Ethylene oxide: used in areas that can be made airtight
- Carboxide: consists of a mixture of ethylene oxide and carbon dioxide; airtight enclosure required
- Sodium hypochlorite solution (household bleach): used for cutaneous exposure diluting two parts bleach to 10 parts water
- Hyamine (benzethonium chloride): very toxic[133][134]

Medical Instrument Decontamination

After an invasive procedure or autopsy is performed, the instruments and area used should be disinfected with a sporicidal agent, such as iodine or chlorine. Peracetic acid may also be used to decontaminate equipment and utensils.[135][136]

Proof of Decontamination

Proof of complete decontamination of soil and vegetation can be difficult because anthrax is a naturally occurring organism. To decontaminate Gruinard Island (Scotland), a mixture of 283 tons of formaldehyde in 2,000 liters of seawater was distributed over the area through a system of pipes. In 1988, fifty years after exposure, Gruinard Island was considered anthrax-free.[137]

Russian Anthrax Compendium

(U/FOUO) This 1981 text entitled "Anthrax" by S.G. Kolesov presents exhaustive information from the height of the Soviet era relating to the disease caused by *Bacillus anthracis*. Kolesov covers epidemiology, microbiology, detection and treatment for this disease. At the time this book was published the Soviet biological warfare program was gearing up for production of ton quantities of anthrax biological warfare agent. As a retrospective text for analysis this book provides additional insights into Russian activities with anthrax. The book is divided into 3 parts [|Part 1] , [| Part 2] ' [| Part 3]

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Anthrax and Botulinum Handbook, 1993

Summary

(U) This notebook consists of a series of tabs providing information on two biologics, **anthrax** and botulinum toxin; and on seven technologies for identification of these biologies.

(U) The tabs provide information at two levels: an executive summary and at a detailed, more technical level of detail. In addition, there is a reference section which contains tabs of useful reference material.

(U) Executive Level

- Tab A presents a summary, in matrix form, describing the capability of each of the seven selected identification technologies to identify **anthrax** and botulinum. A synopsis explains the matrix entries. This tab summarizes the material which follows in Tabs D and E.
- Tab B presents a generic summary of each of the seven identification technologies.
- Tab C presents a detailed write-up on botulinum and anthrax.
- Tab D presents work-ups of literature material in table form on the seven technologies capabilities applied to identification of anthrax. There are two levels of detail: the higher level summarizes literature reviewed for all seven biologies; the next level provides, for each technology cited in the upper level with more than two entries, specific

amplifying information from each literature cite.
- Tab E presents work-ups of literature material in table form on the seven technologies

capabilities applied to identification of botulinum. Tab E contains material at the same level of detail as described above for anthrax in Tab D.

- Tab F provides identification schemes for each biologic which provide both sufficient

and necessary justification for identification of the respective biologic. Detailed. Technical Level

- Tab G provides a detailed explanation for each of the technologies. Tab G is keyed to

items in Tab B.

- Tab H provides detailed abstracted information on each technology when used to identify each biologic; this material was used to construct the tables in Tab D.
- Tab I provides detailed abstracted information on each technology when used to identify each biologic; this material was used to construct the tables in Tab E.
- Tab J contains additional schematic information amplifying Tab F. Tab J also discusses the differences in identification schemes for environmental (dirty) samples vs for laboratory (clean) samples.

The Document is in 2 parts [| Part 1] , [| Part 2]

(U) Non-IC Resources

(U) The Department of Health and Human Services (HHS) is the lead Department for national threats to health, such as anthrax. HHS's components include the Centers for Disease Control, which is the lead agency for public health, the National Institutes of Health, which conducts extensive health-related research, and the Food and Drug Administration (FDA), whose duties include approving potential countermeasures, vaccines, and protective gear that could be used in the event of another anthrax attack.

(U) Please visit these organizations' Wiki pages to learn more about their interest in anthrax and for information on how to contact their fully cleared intelligence interface staff.

Del 19:09, 11 September 2007 (UTC)

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Categories: Pages with reference errors | History of Intelligence | Anthrax | Biological Agents | Epidemiology | Health | Biological warfare | Dictionary | WMD

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