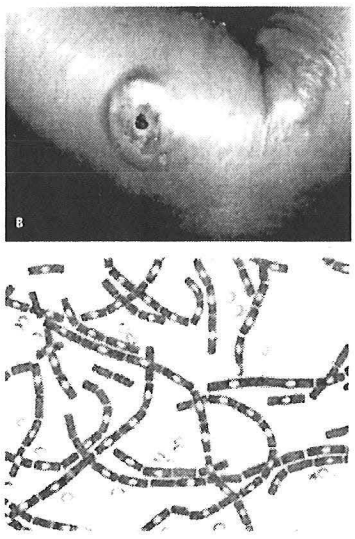
Parkland Memorial Hospital/UT Southwestern Medical Center

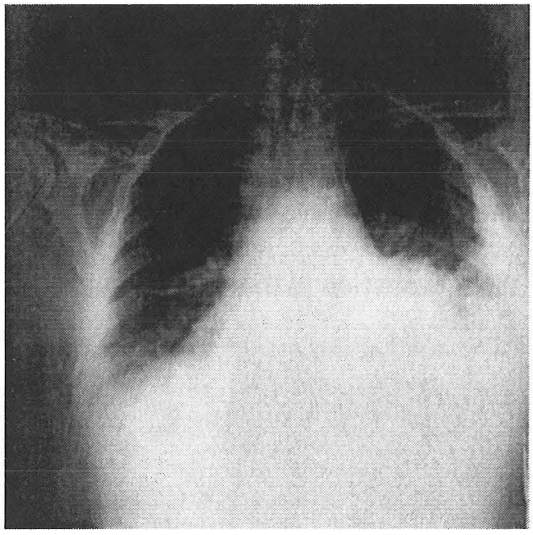
MEDICAL GRAND ROUNDS

October 18, 2001

The Fifth and Sixth Plagues:

Anthrax as a Weapon of Bioterrorism





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This is to acknowledge that Robert W. Haley, M.D., has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Haley will be discussing off-label uses in his presentation.

Biographical Sketch

Robert W. Haley, M.D., is Professor of Internal Medicine and Chief of the Epidemiology Division, Department of Internal Medicine at the University of Texas (UT) Southwestern Medical Center at Dallas and holder of the U.S. Armed Forces Veterans Distinguished Chair for Medical Research Honoring America's Gulf War Veterans. He received his M.D. degree from UT Southwestern Medical School and served an internal medicine residency at Parkland. As a commissioned officer in the U.S. Public Health Service at the Centers for Disease Control and Prevention (CDC) from1973 to1983, he served in the Epidemic Intelligence Service, completed a residency in Preventive Medicine, and directed the Hospital Infections Program. His research interests include epidemiologic research on infectious diseases, outcomes research, and the investigation of unusual illnesses such as Gulf War syndrome. He serves on the Dallas County Medical Society's Board of Health which deals with issues of public health and response to bioterrorism in Dallas County.

Cover Photographs

Clockwise from upper left: typical cutaneous anthrax ("malignant edema") on right arm with black escar and ring of edema extending up the lymphatics; chest x-ray of a typical case of inhalation anthrax ("woolsorter's disease") with widened mediastinum and pleural effusion; microscopic picture of *Bacillus anthracis*, gram positive "box-car" rods in "jointed bamboo" chains.

Security Notice

All of the information in this grand rounds protocol is available on the internet.

In the past five weeks since the September 11 terrorist attacks on the World Trade Center and the Pentagon, the issue of our vulnerability to a terrorist attack involving a biological agent–a bioterrorist attack–has been catapulted to a national obsession. *Time* featured a suburban family outfitted with gas masks, and there have been runs on ciprofloxacin in northeastern cities. Then last week the first case of inhalation anthrax in the U.S. in over 25 years occurred in a photo editor of a tabloid publisher in Florida, followed by isolation of *B. anthracis* from nasal swabs of two of the patient's coworkers. At the end of the week, a case of cutaneous anthrax was identified in Tom Brokaw's executive assistant and another employee in NBC's New York City office.

During this tense period a central question posed by news commentators has been, are we prepared for a bioterrorist attack? Among the many components of being prepared is the ability of practicing physicians quickly to recognize the first cases in such a disaster and to play a productive role in triage, post-exposure treatment and containment of spread of biowarfare-related infections. Since most of these microbial agents are rarely, if ever, seen in Western society, it is widely felt that physicians presently do not have sufficient knowledge and therefore must quickly study the issues to become prepared. This presentation covers anthrax, the agent currently at the top of the news and the one considered the most likely agent to be seen in a bioterrorist attack.[1]

Bioterrorism, A Reality

Pre-1990 View

Before 1990 biological and chemical warfare were considered unlikely threats to western democracies. Throughout history "germ warfare" has rarely been used. The remote instances that came to mind were the catapulting of plague-infected corpses over the walls of besieged cities in the Middle Ages and distributing smallpox- and measles-infested blankets to Indian tribes during the French and Indian War. The rarity of appearances of biowarfare has probably been due to the virtually universal feeling or moral repugnance and technological difficulty. In recent years, however, we have seen the emergence of terrorist groups who are no longer restrained by moral repugnance and who can finance substantial technological development of biowarfare agents.

Iraqi Biological Weapons in the 1991 Persian Gulf War

Although military forces have been concerned about biowarfare throughout the Twentieth Century, the potential for bioterrorist attacks attracted the attention of the civilian public health community after the 1991 Persian Gulf War. Besides a large arsenal of chemical warfare agents employed in the 1980-1988 Iran-Iraq War, in the early 1990s the Iraqi Army was discovered to have large stockpiles of anthrax spores, botulinum toxin, and other agents, loaded into missiles, bombs and other means of delivery. Subsequently, the defection of Ken Alibeck, head of the Soviet Union's biological weapons development activity, confirmed the presence of a massive biowarfare arsenal, including anthrax, smallpox and other agents, which has been poorly guarded since the end of the Cold War. These events stimulated study and organization in public health circles from 1996 to the present.

Brief History of Biowarfare in Modern Times

In WWI the German Army employed plague, cholera, glanders and anthrax in biowarfare attacks on a limited basis. In the 1930s the Japanese Army killed 10,000 in biowarfare

experiments in Manchuria and China In 1942 the British tested anthrax bombs on sheep on Gruinard Island off the northern coast of Scotland. The island remained uninhabitable until decontaminated with formaldehyde in 1987. In 1942 the British secret service assassinated the head of German SS, Hitler's designated successor, with a grenade filled with botulinism toxin. By 1944 the U.S. had 50,000 anthrax bombs ready for use, but never used them.

From 1945 to 1969 the U.S. and Soviet Union secretly developed a wide array of sophisticated biowarfare technology. In 1969 the U.S. unilaterally discontinued development of all chemical and biological warfare. In 1972 the U.S., Soviet Union and others signed the Biological Weapons Convention banning use and development of biological weapons, but no verification procedures or sanctions were included. The Soviet's "Biopreparat," however, continued developing biological weapons secretly. In 1979 an epidemic of deaths from anthrax resulted from an accident in a Soviet BW plant in Sverdlovsk (now Yekaterinburg). In 1980 when all smallpox immunization was discontinued, the Soviets secretly began weaponizing smallpox in four factories in Siberia.

In recent decades every country has Western-trained molecular biologists capable of making any biowarfare agent, though the delivery technology is less available. In the 1991 Gulf War, Iraq had a full arsenal of chemical and biological weapons, as well as efficient, military-grade delivery systems. In the early 1990s the Soviet Union had 55,000 scientists working in biowarfare plants, and now only 30,000 can be accounted for. The rest are said to be available for hire. In 1972 only 4 countries had BW capability; by 1992, 14 did.

The U.S. abandoned biowarfare technology largely because of the high potential for collateral damage, but this is their appeal to terrorists. In addition to the foreign terrorist threat, domestic terrorism has grown in recent years. These include right wing extremists, paramilitary (militia) groups, Puerto Rican terrorists, and other special interest terrorist groups (e.g., anti-abortion extremists). In The Dalles, Oregon, the Rajneeshee cult poisoned salad bars and water supplies with salmonella to reduce voter turnout so they could take over the county government. At St. Paul Hospital in Dallas, a disgruntled microbiology technician purposely contaminated a box of doughnuts with a laboratory stock culture of Shigella dysenteriae and served them to fellow laboratory workers, resulting in several cases of severe dysentery. Bombing of the Oklahoma City federal was carried out by anti-government extremists. Anti-abortion extremists mailed "Anthrax letters" to abortion clinics, although this proved to be a hoax. For several months before attacking the Tokyo and Matsumoto subways with sarin nerve agent, the Aum Shinrikyo religious cult made at least 8 attempts to disperse anthrax spores and botulinum toxin in the streets of Tokyo. No casualties resulted, probably because the anthrax spores were obtained from nonvirulent strains.

Advantages of Biological Weapons

Biological weapons are capable of causing large numbers of casualties with relatively minimal cost, technological expertise, and logistical requirements. For example, the economic cost to produce 50% casualties per square kilometer (1969 dollars) was estimated to be $2,000 for conventional weapons, $800 for nuclear devices, $600 for chemical agents, and $1 for biological weapons.[2]

Cultures of virulent biological agents can be easily obtained from environmental sources, biological supply houses (e.g., the American Type Culture Collection), university or hospital laboratories and clinical specimens. Biological weapons can be directed specifically at human, animal or crop targets. Because of the long incubation period, biological weapons allow time for the perpetrators to escape before the attack is recognized through the initial ill cases.

Disadvantages of Biological Weapons

Biological weapons require careful handling to avoid risk to the perpetrator.[2] They require optimal weather conditions, since aerosolized biologicals may be dispersed by wind currents or thermal updrafts or inactivated by solar irradiation. Anthrax requires substantial biological engineering to ensure that the strain is virulent and to develop a spore preparation and aerosol small enough to reach alveoli.

Criteria for an Effective Biowarfare Agent

From decades of research in developing biowarfare agents, criteria for effective biowarfare agents have emerged. The following are the most important:

- Infective via aerosol
- Stable in aerosol
- High virulence
- Low dose is highly infective
- High titer growth in target
- Low immunity in target population
- Short incubation time but long enough for escape
- High threat force protection
- Target's prophylaxis and treatment are ineffective
- Detection and identification difficult
- High communicability

Biological agents are also compared on their relative efficiency in terms of distance over which an aerosolized dose can remain infective and the number of dead and incapacitated. The following table compares these parameters for an attack on a city of 500,000 population, assuming an optimally engineered weapon.[2]

Agent	Downwind reach (km)	Dead	Incapacitated
Rift valley fever	1	400	35,000
Tick-borne encephalitis	1	9,500	35,000
Typhus	5	19,000	85,000
Brucellosis	10	500	100,000
Q-fever	>20	150	125,000
Tularemia	>20	30,000	125,000
Anthrax	>20	95,000	125,000

Microbes of Choice for Biological Warfare and Bioterrorism

From such comparative criteria, the following are considered the microbial pathogens most suited for use as biological warfare or bioterrorist agents, listed in order of efficiency.[3,4]

- Anthrax
- Smallpox
- Plague
- Botulism
- Q-fever
- Tularemia
- Brucellosis
- Influenza
- Venezuelan Equine Encephalitis (VEE)
- Hemorrhagic fevers (Marburg, Lassa viruses)
- Ebola virus
- Yellow fever virus
- Typhus
- Meliodosis

Epidemiologic Case Reports

Case Report #1: Florida and New York City, October 2001

On October 2, 2001, a 63-year old photo layout artist for a national tabloid publishing company was hospitalized in Lantana, Florida, with high fever, altered consciousness, abdominal pain, pleural effusions, meningitis and suspected sepsis. The diagnosis of inhalation anthrax was suspected from a gram stain of cerebrospinal fluid, and within two days it was confirmed by positive blood cultures. Despite antibiotics and supportive care, on the third hospital day the patient died of respiratory failure and circulatory collapse. Since no other cases had been identified, investigation by the local health department and the U.S. Centers for Disease Control and Prevention (CDC) initially centered on reconstructing the patient's recent exposure and travel history for the past 60 days and maintaining surveillance, including nasal cultures, of relatives and others who had been in close contact with the patient. The investigation included viting places the patient had visited during a trip to North Carolina, taking environmental samples for culture from the patient's home, contacting local veterinarians regarding possible anthrax in animals, setting up surveillance for additional cases in local emergency rooms and medical clinics, and sending the organism to the Los Alamos National Laboratory in New Mexico for DNA fingerprinting and comparison with a geographical catalog of anthrax strains. On October 7, the patient died.

On October 8 a 73-year old man, who worked as a mail supervisor at the same office, was found to be asymptomatically colonized with *B. anthracis* by nasal swab culture, and an environmental culture taken from the first patient's computer keyboard was positive for *B. anthracis*. CDC investigators focused the investigation on the 790 employees who worked in the publisher's office building, taking nasal swabs for culture and acute serum for ELISA, and instituting prophylaxis with ciprofloxacin. Simultaneously, the FBI declared the incident a criminal case and began its investigation. Subsequently, the nasal swab of one additional employee was positive, and an ELISA on acute sera was positive in five additional employees. No further cases of illness appeared among the employees. The strain of B anthracis proved to be sensitive to penicillin and tetracycline, practically ruling out one of the highly virulent, engineered strains developed for biowarfare.

Following the initial announcement, the FBI received numerous reports of people opening mail containing powder, but most proved to be hoaxes. However, on September 25 the administrative assistant of an NBC television network ancor opened an envelope containing a threatening letter and a white powder and subsequently developed fever and a skin lesion from which *B. anthracis* was cultured. She was treated with ciprofloxacin and recovered uneventfully. Around October 13 another NBC employee who had handled the suspect letter developed an undisclosed illness which was diagnosed as anthrax and was treated with ciprofloxacin. Cultures of the suspicious letter were positive for *B. anthracis*. A suspicious letter containing pornagraphic literature, received by an employee of the Microsoft Corporation, in mid-October also tested positive for *B anthracis*.

Case Report #2: Egypt 1250 B.C.

[The Fifth Plague] Then the Lord said to Moses, "Go in to Pharaoh, and say to him 'Thus says the Lord, the God of the Hebrews, "Let my people go, that they may serve me. For if you refuse to let them go and still hold them, behold, the hand of the Lord will fall with a very serve plague upon your cattle which are in the field [all your grazing animals], the horses, the asses, the camels, the herds, and the flocks. But the Lord will make a distinction between the cattle of Israel and the cattle of Egypt, so that nothing shall die of all that belongs to the people of Israel."'" And the Lord set a time, saying, "Tomorrow the Lord will do this thing in the land." And on the morrow the Lord did this thing; all the cattle of the Egyptians died, but of the cattle of the people of Israel not one died. And Pharaoh sent, and behold, not one of the cattle of the Israelites was dead. But the heart of Pharaoh was hardened, and he did not let the people go.

[The Sixth Plague] And the Lord said to Moses and Aaron, "Take handfuls of ashes from the kiln, and let Moses throw them toward heaven in sight of Pharaoh. And it shall become fine dust over all the land of Egypt, and become boils breaking out in sores on man and beast throughout all the land of Egypt." So they took ashes from the kiln, and stood before Pharaoh, and Moses threw them toward heaven, and it became boils breaking out in sores on man and beast. And the magicians could not stand before Moses because of the boils, for the boils were upon the magicians, and upon all the Egyptians. But the Lord hardened the heart of Pharaoh, and he did not listen to them; as the Lord had spoken to Moses.

Exodus 9:1-12
Rev. Std. Version

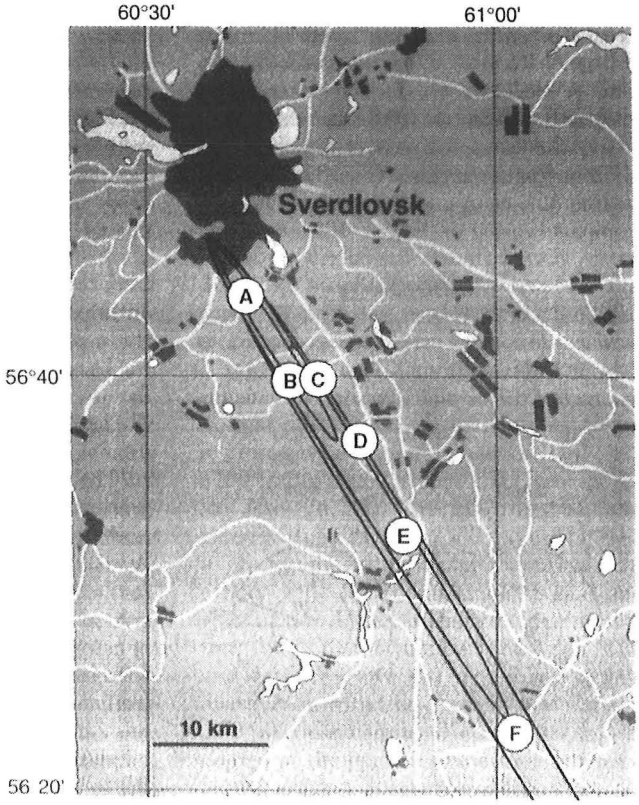
Case Report #3: Sverdlovsk, Soviet Union, 1979 A.D.

Within a 43-day period 79 cases of human anthrax occurred and 68 people died in Sverdlovsk, Soviet Union (now Yekaterinburg), an industrial city of 1.2 million people[5] (see figure). The Soviet government attributed the epidemic to gastrointestinal anthrax from consuming poorly cooked meat. In the early 1990s after the fall of the Soviet Union, American and Russian pathologists reviewed the retained pathologic specimens and found clear evidence of inhalation anthrax. Geographical analysis showed the cases tightly clustered in a linear plume southeast of the city, and meteorologic analysis pinpointed the dated of exposure as 2 April 1979 (see figures on the next page). The incubation period varied from 1 to 43 days. The mortality rate was high regardless of the date of onset.

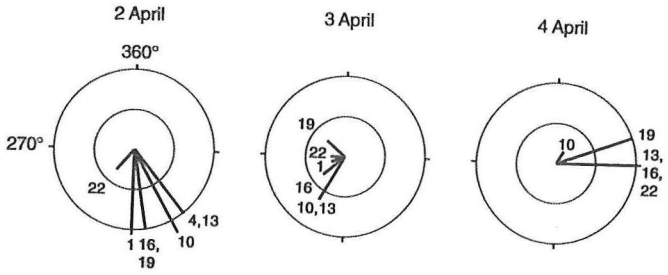
Subsequently the Yeltsin government admitted a mishap at a biological weapons plant in the city at the predicted location and date. A worker at the Sverdlovsk biological weapons production plant had come to work on a weekend and forgotten to turn on the emission filtration device. Liberation of less than a gram of anthrax spores had caused illness and death up to 40 km away. From the self-limited time course of the epidemic in the absence of widespread immunization (only 47,000 of the 1.2 million were immunized), all cases were thought to have resulted from primary aerosolization from the initial event rather than secondary aerosolization

from spores remaining in the environment. Interestingly, the youngest case was 24, suggesting diminished susceptibility to inhalation anthrax in children.

Probable daytime locations on 2 April 1979 of 66 patients with inhalational anthrax. Countour lines represent estimated zones of constant dosage of airborne exposure from the military microbiology facility. Sixty-two of the 66 patients were in the zones of exposure. (From Meselson et al.[5])



Villages reporting epidemics of animal anthrax between 5 and 10 April 1979. Contour lines indicate zones of constant dosage of airborne exposure from the military microbiology facility. (From Meselson et al.[5])

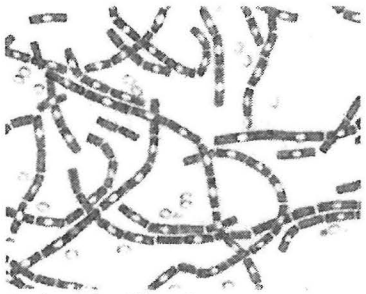


Wind directions and speed reported from Koltsovo airport for the period 2 to 4 April 1979. Numbers at the downwind end of each line are local standard times. Inner and outer concentric circles designate wind speeds of 2.5 and 5.0 m s^{-1}, respectively. Zero wind speed was reported for 0400 on 3 April and for 0100 on 4 April. No data were reported for 0700. (From Meselson et al.[5])

Anthrax, The Organism and the Disease

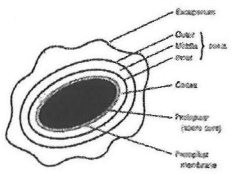
The Organism

Bacillus anthracis, the etiologic agent of anthrax, is a plump gram-positive rod with squared ends ("box car bacilli") that appears in long "jointed bamboo" chains microscopically. Robert Koch first demonstrated transmissibility of an infective agent with this organism, thereby establishing Koch's Postulates.[1,6,7]



Anthrax bacilli and spores

Each bacterial form produces one endospore, which is the infective particle. Metabolically, *B. anthracis* is an aerobe or facultative anaerobe. The vegetative forms tend to multiply in the presence of adequate nutrition supply and sporulate when nutrient supply is exausted, as in the carcass of a decaying animal, but sporulation also requires oxygen. Spores are lighter than air and can remain suspended in air for hours, allowing infection many miles downwind from the release point. Under the right conditions spores in the carcases of dead animals can survive in soil, remaining infective for decades ("anthrax grave").



Anthrax endospore (from Turnbull[7])

In culture *B. anthracis* forms characteristic comma-shaped colonies. Spores formed on culture plates constitute an extreme hazard to laboratory workers.

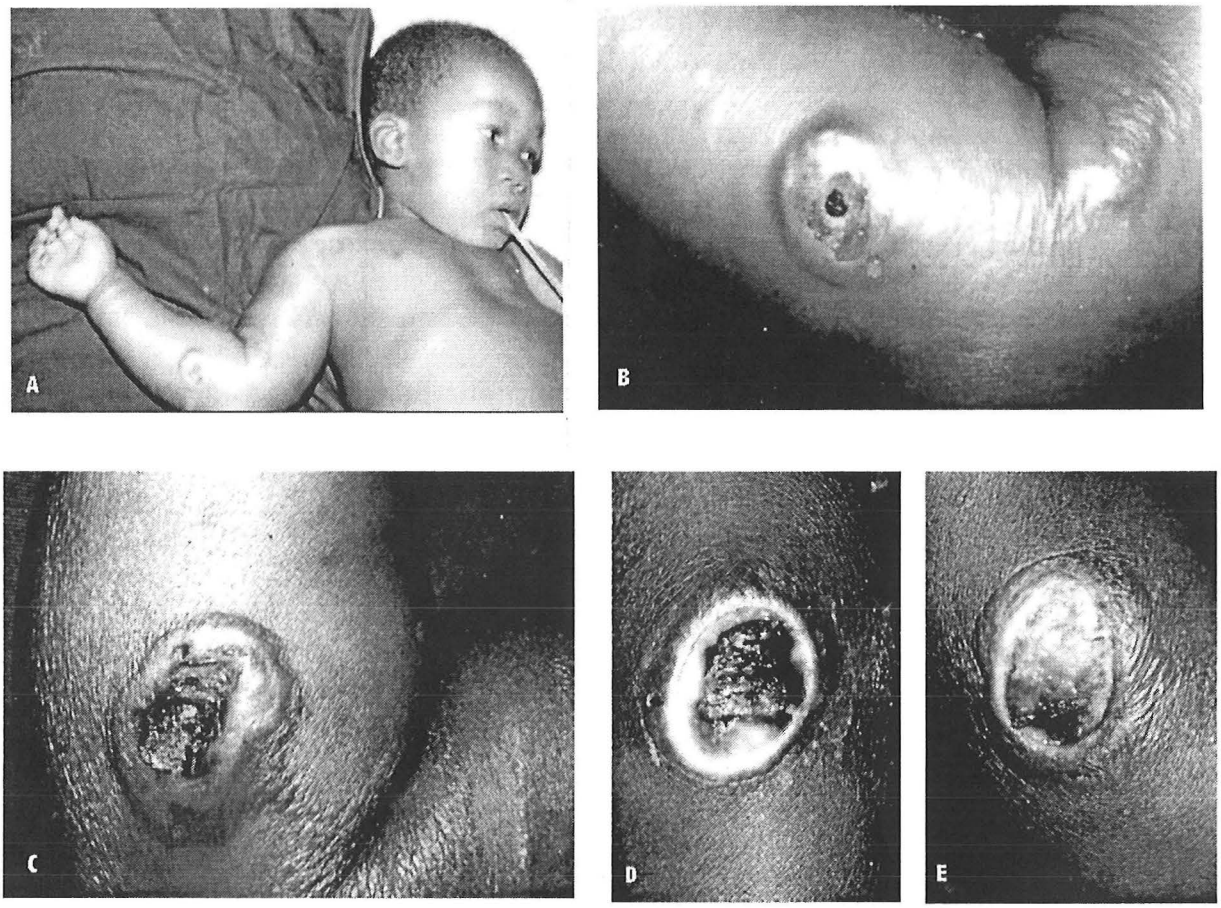
The virulence of *B. anthracis* is determined by two factors, both of which must be present: 1) a thick polypeptide capsule that protects it from phagocytosis, and 2) a complex exotoxin that causes edema and lethality and further protects from phagocytosis. These virulence factors are encoded on two plasmids. The pXO1 plasmid codes for the genes that make the exotoxins, and the pXO2 plasmid codes for the thick capsule. Strains lacking either plasmid are avirulent.

Three Clinical syndromes

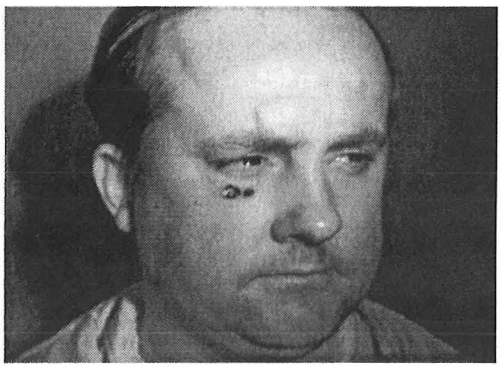
Anthrax produces three important syndromes. They are *cutaneous anthrax*, *gastrointestinal anthrax*, and *inhalation anthrax*.[6,8] In the U.S. there are an average of less than one case of cutaneous anthrax per year and rare cases or clusters of gastrointestinal or inhalation anthrax.

Cutaneous Anthrax occurs when anthrax spores get into tiny cuts in the skin. After limited germination produces edema and necrosis in the cutaneous site, most spores are engulfed by macrophages which transport them to regional lymph nodes but do not kill them. Inside macrophages, the spores germinate to the vegetative bacillus form, which elaborate the exotoxins.

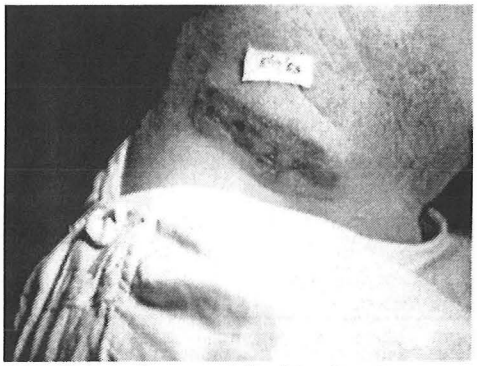
Clinically, the lesion begins as an innocent-looking cutaneous papule. Within 2 days it becomes a 1-cm nonpurulent, *nonpruritic* vesicle, typically ringed by a remarkable amount of edema in the surrounding skin (see photographs on next page). The ring of edema, which usually extends up the lymphatics, should strongly suggest the diagnosis. Within a week, the vesicle ruptures, leaving a characteristic black escar surrounded by the ring of edema. Historically, the black escar gave rise to the name of the disease ("anthrax" comes from the Greek word for coal *anthrakis*). In 5-30% of untreated cases, the cutaneous infection invades the blood stream, producing metastatic infections in the meninges and GI tract and death from respiratory failure and circulatory collapse. However, appropriate antimicrobial treatment should prevent death in all cases, although it does not alter the natural progression of the skin lesion.



Evolution of an escar from cutaneous anthrax ("malignant edema") in a 4-year-old boy. (A&B) the lesion when first seen (day 0). Note the arm swollen from the characteristic edema extending up the lymphatics. (C) Day 6. (D) Day 10. (E) Day 15. Although penicillin treatment was begun immediately and the lesion was sterile by about 24 hours, it continued to evolve and resolve as seen. (Photographs supplied by W.E. Kobuch, M.D., St. Luke's Hospital, Lupane, Bulawayo, Zimbabwe, and obtained from Turnbull[7]).



Cutaneous anthrax under right eye



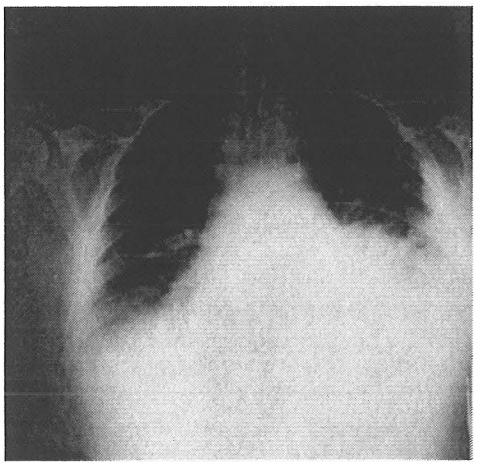
Cutaneous anthrax on right side of neck

Gastrointestinal Anthrax occurs when the spores are ingested in contaminated, undercooked beef or other types of meat. The spores germinate in macrophages, which transport them to regional lymph nodes. There they further multiply, producing edema, necrosis and lymphatic obstruction.

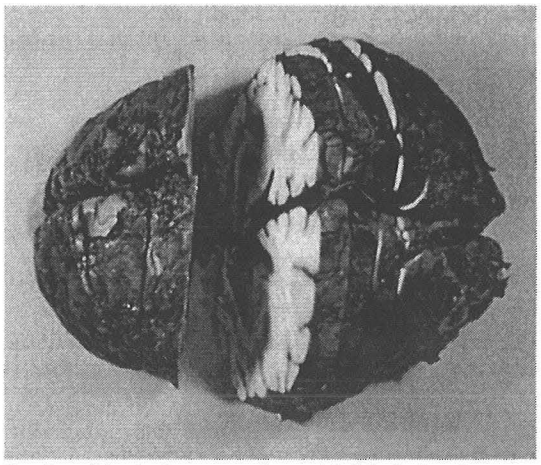
Clinically, gastrointestinal anthrax can present as oropharyngeal or intestinal disease. Typically1-7 days after ingestion of poorly cooked, antrax-contaminated meat, patients develop fever and abdominal pain, followed by nausea, vomiting and diarrhea. Lymphatic obstruction and toxin production cause GI tract edema, turbid or hemorrhagic peritoneal fluid, and GI hemorrhage. Untreated, some cases result in sudden death from circulatory collapse due to volume loss and toxin-related shock. Appropriate antibiotic treatment may reduce the mortality rate to 50%.

Inhalation Anthrax, the form expected in a bioterrorist attack, occurs when large numbers of anthrax spores are inhaled with ambient air. The spores (1 μm in diameter) are inhaled into the alveoli. Most spores are engulfed by macrophages and transported to hilar lymph nodes. There they may remain dormant for up to 60 days (usually 1-6 days), accounting for the variable incubation period of illness. When germination finally occurs, the vegetative forms rapidly multiply and elaborate toxins, leading to necrosis of the lymph nodes, massive edema of the mediastinum and chest wall, and hemorrhagic mediastinitis. Lymphatic or hematogenous spread to the meninges, gastrointestinal tract, liver and spleen occur in up to half of cases. Death from respiratory failure and circulatory collapse often occurs suddenly when the macrophages begin rupturing, rapidly dumping large numbers of organisms and amounts of lethal toxin into the circulation.

Clinically, patients are unaware of inhaling the spores. After a highly variable incubation period, usually 1-6 days but extending out to 60 days, patients first experience a mild flu-like illness with low grade fever, malaise, myalgias, cough and mild chest pain. After a day or two, these prodromal symptoms often disappear. After a 3-5 day quiescent period, symptoms reappear suddenly with high fever, severe chest pain, shock, respiratory failure and often a rapid death. In the final stage, patients may have hemorrhagic meningitis (50% of patients), marked chest wall edema, and pleural effusions, but frank pulmonary infiltrates are unusual.



Chest x-ray of inhalational anthrax 20 hours before death. Note widened mediastinum, pleural effusion and absence of an infiltrate. (From P.S. Brachman)



Sectioned brain from a patient with inhalational anthrax complicated by hemorrhagic meningitis. Note the blood staining over the entire surface of the brain.

Infective Dose

Based on experiments in primates, the LD50 (lethal dose in 50% of those exposed) for inhalation anthrax in humans is estimated to be between 2,500 and 55,000 spores inhaled.[1] This depends heavily, however, on particle size of the aerosol inhaled. A batch of anthrax spores produced by crude methods would be expected to have spore aggregates too large to reach the alveoli where the disease is produced. Spore preparations capable of causing inhalation anthrax in large numbers of people would be expected only from terrorists supplied by one of the countries with sophisticated biowarfare plants capable of manufacturing extremely fine particle aerosols. This is the limiting factor in the number of casualties in an anthrax attack.

Anthrax spores cannot cause cutaneous infection in intact skin but gain entrance through a skin abrasion or fissure. For cutaneous anthrax particle size of the infective exposure is not as important.

Incidence of Anthrax Infection

From 1900 to 1978 only 18 cases of inhalation anthrax are known to have occurred in the U.S., and no case had occurred since 1978 until the October 2001 case in Florida. Of the cases before 1978, the source was presumed to be imported goat hair in nine, occupational exposure in tanneries in three, imported wool in one, imported wool rugs in one, and occupational exposure in bacteriology laboratories in two.[1,8] The source was unknown for two. Consequently, the occurrence of inhalation anthrax in a person without an obvious occupational exposure is a singular occurrence and cause for alarm.

Although cutaneous anthrax has been far more common than inhalation anthrax in the U.S., the yearly incidence of cutaneous anthrax in humans has declined dramatically over the Twentieth Century from approximately 200 cases per year in early years to less then one case on average per year today.[9] Such cases are almost always acquired from obvious contact with animals or animal products.

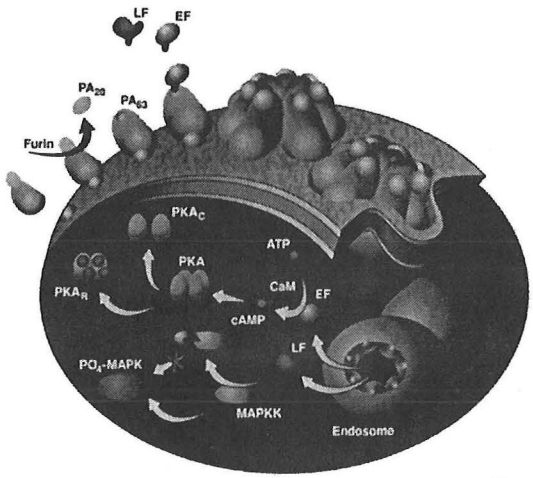
Gastrointestinal anthrax has not been reported in the U.S., with the possible exception of a small cluster that occurred in August 2000, when six members of a family in rural Minnesota developed mild gastroenteritis from well cooked hamburger from an anthrax-infected cow.[10]

Pathogenesis of Toxin Effects

The anthrax toxin is composed of three separate proteins elaborated by the vegetative bacterial form. These are *Protective Antigen* (PA), *Edema Factor* (EF) and *Lethal Factor* (LF).[1,6,11] PA was named for the fact that injecting it into guinea pigs protects them from anthrax infection. EF and LF are named for their pathophysiologic effects. The combination of PA and EF is referred to as *Edema Toxin*, and the combination of PA and LF, as *Lethal Toxin*.

PA binds to the cell membrane and facilitates the entry of the other two factors into the host cell by the endosomal pathway.[11] Once inside the cell, EF facilitates conversion of ATP to cAMP, and the excess cAMP stimulates massive edema formation, one of the early, characteristic features of the clinical illness. EF inhibits neutrophil function as well.

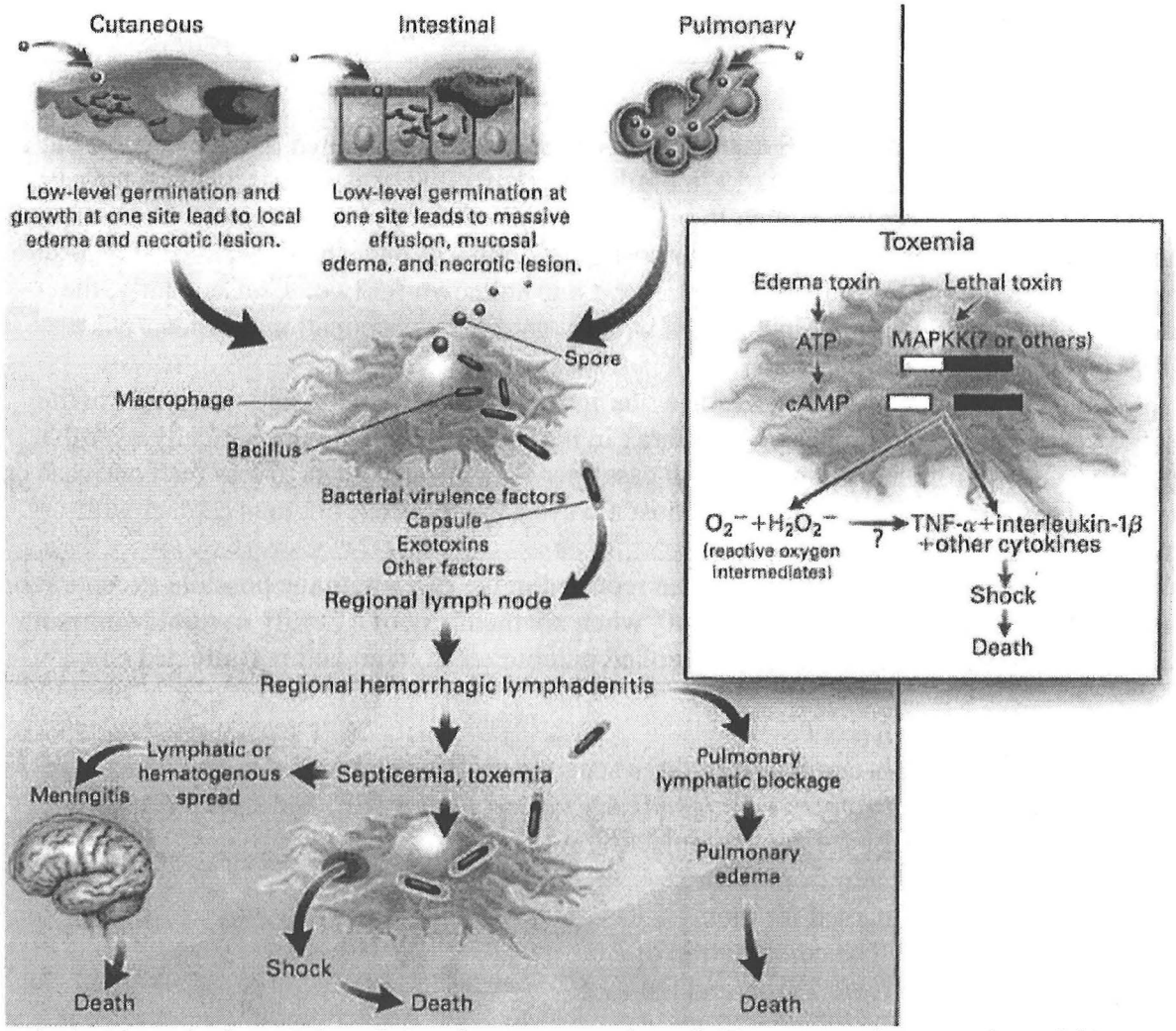
When LF gains entry to the cell, it acts as a



Anthrax toxin activity (from Duesbery and Woude[11]).

metalloprotease, cleaving the amino terminus of mitogen-activated protein kinase kinases 1 and 2 (MAPKK1 and MAPKK2).[11] By an unknown mechanism, this stimulates the production of tumor necrosis factor (TNF-α) and interleukin-1β, which in turn cause tissue necrosis, respiratory failure, circulatory collapse and death.

Lethal toxin is the dominant virulence factor of B anthracis and is responsible for the terminal circulatory collapse in the death of infected animals and humans.[11,12] Early in the course of fatal infections the concentration of bacilli progressively increase inside macrophages and in the blood, while the lethal toxin does not appreciably increase in blood. Suddenly large numbers of macrophages burst, releasing bacilli and large amounts of lethal toxin into the circulation. Death ensues within hours.



Pathophysiology of anthrax. Pathogenic *B. anthracis* spores reach aveoli, GI mucosa or subcutaneous layer of skin. Low-level germination at the primary site causes local edema and necrosis. Spores are phagocytosed by macrophages and germinate. Macrophages migrate to regional lymph nodes. Vegetative bacilli grow in the lymph node, creating regional hemorrhagic lymphadenitis. Bacteria spread through the blood and lymph and increase to high numbers, causing severe septicemia. High levels of exotoxins cause overt symptoms and death. In 50% of cases of inhalational anthrax, meningeal involvement occurs by lymphatic or hematogenous spread. Late, peribronchial hemorrhagic lymphadenitis blocks pulmonary lymphatic drainage, leading to pulmonary edema. Death results from septicemia, toxemia, or pulmonary insufficiency. The inset shows the effects of anthrax exotoxins on macrophages. (From Dixon et al.[6]).

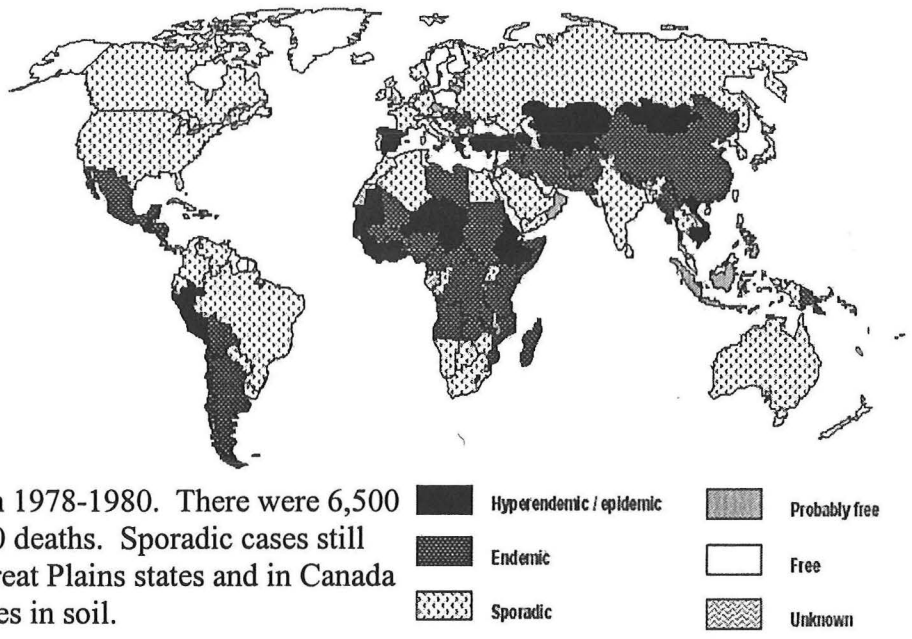
Individual Variation in Susceptibility to Anthrax Infection

Although there is no direct evidence of individual variation in susceptibility to anthrax infection, substantial variation is suggested by indirect evidence. While large numbers of people work with anthrax-infected animals and animal products, relatively few ever develop clinical anthrax infection.[1] In the Sverdlovsk epidemic, tens of thousands of people were potentially exposed to weapons-grade anthrax spores, and yet only 79 developed disease.[1,5] The lack of cases in children in the Sverdlovsk epidemic and other evidence suggests that children may have uniquely low susceptibility. While the evidence is meager and indirect, it is possible that a relatively small proportion of the population is susceptible to anthrax infection, and that the projected numbers of casualties of an anthrax bioterrorist attack have been exaggerated. On the other hand, excessively large numbers of inhaled spores of a highly virulent weapons-grade strain might be sufficient to overwhelm such protective factors. At any rate, public health planning must include worst-case estimates to ensure readiness.

Worldwide Occurrence of Anthrax Infection

Zoonotic anthrax occurs sporadically throughout North America, the northern half of South America, Europe, Russia, India and Australia, and at epidemic or hyperendemic levels in Mexico, Central America, southern and western South America, and most of Africa and Asia[13] (see world map). Consequently, anthrax organisms are readily available in nature.

Widespread animal vaccination programs have virtually eliminated both animal and human anthrax in developed countries but it is still a problem in developing countries. A large epidemic occurred in farm animals and humans in Zimbabwe during the Rhodesian civil war in 1978-1980. There were 6,500 human cases and over 100 deaths. Sporadic cases still occur in animals in the Great Plains states and in Canada due to persistence of spores in soil.



Modes of Spread

Anthrax is primarily an epizootic disease of grazing animals (herbivors), particularly sheep, goats, cattle and rarely swine.[13] Animals most commonly get gastrointestinal anthrax during outbreaks from grazing on plants contaminated with anthrax spores. Some of the infected die when the vegetative bacterial forms and the toxin spread to the blood stream. The decomposed carcasses of infected animals may produce huge numbers of spores that blow onto soil and plants to continue the zoonotic cycle. Heavy rains and wind often concentrate spores in low lying areas. Spores remain infective longer in alkaline soil and are suppressed by acidic soil.

Rarely the unearthing of the carcass of a long-dead animal ("anthrax grave") will initiate

an epizootic in an area where anthrax had been eradicated. Biting insects (e.g., tabanid flies and blow flies) serve as important modes of transmission among certain animal species in some locales.[13] Foodborne outbreaks have been traced to use of contaminated bone meal as cattle feed. This well known hazard led decades ago to the ban on bone meal feed that prevented transmission of bovine spongiform encephalopathy (BSE) in the U.S. However, bone meal containing recycled animal parts remains a common practice worldwide.

Control measures include universal vaccination of grazing animals in areas where anthrax is active and burning of the carcasses of animals dying of anthrax. When the disease has been eradicated in a region, animal vaccination is usually discontinued and surveillance for new cases is maintained. Burying of anthrax-infested carcasses is not recommended because this creates a potential reservoir of spores that may remain infective for decades. Anthrax tends to break out in countries disrupted by war when veterinarian services and disease surveillance degenerate.

Humans tend to get anthrax in several settings.[8-10,14,15] Cutaneous anthrax is generally acquired by farm hands (e.g., herders, butchers, slaughterhouse workers) exposed to farm animals or by tourists or others who work with or receive woolen goods from endemic countries. Inhalation used to be an occupational hazard of workers handling sheep and goat hair and skins ("woolsorter's disease"). Finally, microbiology laboratory technicians who process bacterial cultures may develop inhalation anthrax by opening and inadvertently breathing or blowing on a culture plate growing anthrax.

Persistence of Spores in the Environment

The fact that anthrax spores may under ideal conditions persist in the environment for decades, and the well documented hazards of "anthrax graves," have given rise to concerns of continuing long-term infection from *secondary aerosolization* following a bioterrorist attack with anthrax. Pasteur first raised concerns over "permanent ground contamination;" however, experts in epizootic anthrax contend that this fear is exaggerated.[13] The residual threat is primarily to grazing animals and is determined by the initial concentration of spores in soil and soil conditions (e.g., high pH, high temperature, moisture, organic nutrition), generally lasting "three months to three years."[13]

During WWII the British exploded anthrax bombs on Gruinard Island, off the northern coast of Scotland, resulting in immediate infection of sheep grazing nearby and heavy contamination of soil with anthrax spores; however, no later anthrax infections are known to have resulted from the Gruinard anthrax contamination. By the early 1980s, spores could still be detected in small areas near the detonation site. These were decontaminated with formaldehyde in sea water, and sheep grazed for 4 months without developing disease (although infectivity of the soil was not tested with sheep before decontamination).[16,17]

In the Sverdlovsk epidemic all of the human anthrax cases (longest incubation period 43 days) were attributed to primary aerosolization, and no cases were thought to occur by secondary aerosolization.[5]

Due to the relatively large dose of spores required to produce human airborne infection, inhalation anthrax via secondary aerosolization following a bioterrorist attack seems an unlikely hazard for humans. Decontamination of heavily spore-contaminated soil, if required, may be accomplished with 4% formaldehyde, 3% peracetic acid, or 5% glutaraldehyde in water.[16]

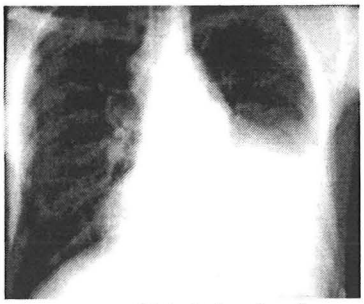
Differential Diagnosis of Inhalation Anthrax

The differential diagnosis of inhalation anthrax, the syndrome of most interest from the bioterrorist standpoint, includes other conditions that could present with sudden circulatory

collapse in conjunction with a chest x-ray showing a widened mediastinum and possibly a pleural effusion but no infiltrate, after a recent mild flu-like prodrome. Conditions most likely to mimic this include ruptured aortic aneurysm, lymphoma or sardoidosis with sepsis, acute bacterial mediastinitis, and mycoplasmal pneumonia.[6] Less likely possibilities include Legionnaires' disease, psittacosis, tularemia, Q fever, viral pneumonia, histoplasmosis with mediastinal fibrosis, superior vena cava syndrome, coccidiodomycosis and silicosis.

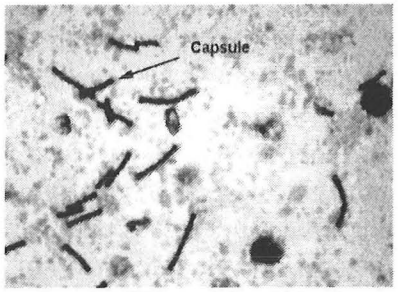
Diagnostic Methods

Inhalation Anthrax. *A chest x-ray with widened mediastinum in a previously healthy person presenting with a flu-like illness (the prodrome) or a severe septic illness (the final stage) should be considered inhalation anthrax until proven otherwise.* One such case is highly suggestive of a bioterrorist attack unless another source of infection, such as recent animal exposure, can be documented. Additional cases in the same locale confirm belligerent intent and provide epidemiologic information for identifying the circumstances of exposure.



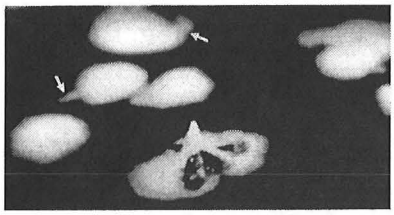
Chest x-ray of inhalational anthrax.

The diagnosis of anthrax can be supported most rapidly by demonstrating the characteristic "box car" gram positive rods in chains on gram stain of fluid from a skin lesion, sputum, pleural fluid, cerebrospinal fluid or peritoneal aspirate.[18] Organisms may also be seen on a standard peripheral blood smear in the advanced stages when large numbers of organisms invade the bloodstream. At least one of these specimens should be positive in virtually every case. A false positive gram stain could result from insufficient decolorization of material from an infection with gram negative rods.



Anthrax bacilli in peripheral blood smear

Standard bacterial culture of the same clinical specimens confirms the diagnosis, but may be negative in the prodromal phase. *B. anthracis* grows rapidly on blood agar and other standard media, and cultures are usually positive within 18-24 hours.[18]



Colonies of *B. anthracis* on blood agar. Notice the typical comma-shaped colonial morphology (arrows).

More sensitive ELISA and PCR tests are available at special reference centers, including CDC, but are useful primarily for late verification of the diagnosis or for evaluating hoaxes.

Asymptomatic exposure to anthrax spores may be detected by culturing a standard nasal swab on blood agar. Demonstration of a rise in ELISA titer from acute to convalescent sera differentiates a true infection from non-infective exposure to spores.

Cutaneous Anthrax. The diagnosis is usually made from the clinical appearance of a typical coal-black escar surrounded by a ring of edema and is confirmed by gram stain or culture of fluid from the escar or biopsy of the escar.

Environmental Detection. Powders (e.g., in a letter) suspected of being anthrax spores should immediately be sealed in a plastic bag, and the public heath and law enforcement and public health system activated by calling 911.[15] Potentially exposed persons should wash hands thoroughly and participate in medical followup. Spores consistent with *B. anthracis* can be

presumptively identified by phase microscopy or direct fluorescent antibody testing. Definitive identification is by culture on blood agar. If initial samples are positive for *B. anthracis*, additional environmental sampling can be done with Rodac plates, standard swabs and air sampling devices. Residual environmental contamination with anthrax spores can be decontaminated with a solution of one part household bleach to 10 parts water.[15] There are currently no practical methods for rapidly detecting clouds of anthrax spores in air.

Strain Identification. DNA fingerprinting of an anthrax strain may be epidemiologically useful in understanding the source and history of the involved strain.

Prevention

In the 1870s Robert Koch demonstrated the bacterial origin of anthrax ("Koch's postulates") and discovered the spore stage that persists in the environment.[19] In 1880 John Bell recognized *B. anthracis* as the cause of woolsorter's disease and eliminated it in wool factory workers by disinfecting the wool before processing. In 1880 William Green first successfully immunized livestock, and in the following year Louis Pasteur repeated it with a different regimen which has been used ever since with slight modification.

Three anthrax vaccines, developed in Russia, the U.S. and Britain, respectively, are in use for immunizing humans.[19] The vaccines are produced by growing nonvirulent (unencapsulated) strains of *B. anthracis* in vats and decanting off and treating the cell-free filtrate for use as the vaccine. The older Russian vaccine was proven to be highly protective against inhalation anthrax, but the American and British vaccines have not been thoroughly evaluated. The current U.S. vaccine is produced in only one factory (Bioport, Inc, Michigan), but substandard manufacturing processes caused it to be closed by the FDA pending restructuring of the production processes. After a controversial campaign to immunize all members of the U.S. military, only small amounts of the vaccine remain, and it's use is problematic.

Treatment recommendations

Antimicrobial prophylaxis before exposure and treatment in the prodromal period are highly effective in preventing death from all three forms of anthrax but is generally not successful if delayed until the final stage of the illness.[1,20] Since inhaled spores transported to mediastinal lymph nodes can begin germinating from 1 to 60 days after the initial exposure, antimicrobial prophylaxis must be continued for 60 days or until 2 doses of anthrax vaccine can be administered.[1] If anthrax vaccine is available, doses should be administered at 0, 2 and 4 weeks, and the patients may be taken off antibiotics at 30 days. Regardless of the duration of antibiotic treatment, the antibiotic should be discontinued under medical supervision in case of recrudescence.[1]

In a potential bioterrorist event, penicillin and tetracycline resistance should be assumed initially. In such a situation ***ciprofloxacin*** *(or other fluorquinolones such as levofloxacin or ofloxacin) is the drug of choice for empirical treatment and prophylaxis, pending antimicrobial susceptibility test results.*[15] Ciprofloxacin is highly effective against all known strains of anthrax and has a very benign side effect profile. FDA recently approved ciprofloxacin for anthrax prophylaxis and treatment.

For sporadic cases with penicillin-tetracycline-sensitive strains, penicillin and doxycline are the FDA-approved drugs of choice for all three anthrax syndromes and for prophylaxis.[1] Since resistance to these agents occurs uncommonly in nature, antimicrobial susceptibility testing should guide the choice of antibiotics. Resistance to penicillins (β-lactamase-producing strains) and tetracyclines occur in nature and are likely to be found in an anthrax strain used by

an organized bioterrorist organization. In fact, finding a strain to be sensitive to penicillin and tetracycine virtually rules out one of the highly virulent strains engineered for biowarfare.

Many other antibiotics may also be effective against anthrax and should be tried in case of mass infection that exhausts the ciprofloxacin supply or when antimicrobial susceptibility results on the epidemic strain become available. Other good choices where ciprofloxacin is in short supply would include the *other fluoroquinolones, chloramphenicol, clindamycin, β-lactamase-resistant and extended-spectrum penicillins, macrolides, aminoglycocides, vancomycin, cefazolin and the other first-generation cephalosporins.*[1] Almost all strains of *B anthracis* are innately resistant to trimethoprim-sulfamethoxazole and the second and third generation cephalosporins, and these should not be tried.[1]

Intravenous antimicrobial infection is generally indicated for symptomatic anthrax infection, although in a mass epidemic situation oral therapy would have to suffice.[1] Oral therapy is recommended for prophylaxis in asymptomatic exposed persons.

Ciprofloxacin and the other fluoroquinones are generally contraindicated in children <18 and in pregnant women due to a potential for arthropathy in developing cartilage. These contraindications, however, would generally be disregarded in a bioterrorist anthrax threat due to the overriding risk of death from inadequately treated anthrax infection.[1] In such an event, ciprofloxacin should be given initially and the agent changed to penicillin or another agent when results of antimicrobial susceptibility testing become available.

Corticosteroids can be used to treat several edema often seen in severe anthrax infection.

A Bioterrorist Attack with Anthrax

Although several countries are known to have weapon-grade anthrax for biowarfare and Iraq had artillery shells and bombs filled with anthrax spores during the 1991 Gulf War, no large-scale biological weapons attack involving anthrax has ever been reported. If one were to occur, due to the technical difficulty of producing an anthrax spore preparation of small enough particle size and delivering it effectively, *the most likely scenario of such an attack would involve serious illness in relatively few people.* This is typified by the October 2001 anthrax exposures in Florida and New York. Such an occurrence, though likely to cause widespread anxiety, would be relatively easily managed by public health and medical resources currently in place.

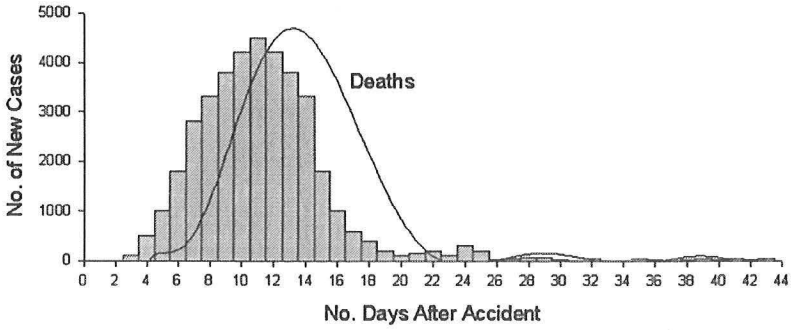
There is, however, a plausible doomsday scenario, which, though highly unlikely, would cause tens of thousands of life-threatening cases of inhalation anthrax. Such an attack would quickly overwhelm current healthcare resources, and some lives would inevitably be lost. Pre-event planning and timely action by public health and medical resources, however, would greatly reduce the numbers of infections and deaths.

What would an epidemic be like without a public health response?

A vivid description of the 1918 influenza epidemic in Philadelphia provides a vivid description of what it would be like to confront a massive epidemic of a fatal disease.[21] In mid-September the first people became ill. The next day a sailor died, followed shortly by a civilian death. The next day 20 people died, including a nurse who had cared for the first sailor, and the daily death toll continued to climb thereafter. By September 26, all the city's hospitals were full and closed to further admissions, but the numbers of new cases was beginning to decrease, suggesting that the epidemic would end. On September 28, however, against the advice of local physicians, the mayor declined to cancel a war bonds parade. Within two days after the parade, an estimated 160,000 Philadelphians developed influenza. Shortly the death toll climbed into the hundreds per day, reaching 4,597 in one weak at its peak. Finally responsible medical leadership emerged and took measures to curtail spread, and the epidemic rapidly subsided. During the six week period of the epidemic, at least 12,600 deaths occurred in the city.

The following is a possible day-by-day scenario, under the worst-case assumptions: a) weapons-grade anthrax spores were used, b) 50,000 of 200,000 citizens in the path of the wind-driven aerosol developed inhalation anthrax, c) the distribution of cases would follow the epidemic curve of the 1979 Sverdlovsk epidemic, and d) *no effective public health response was mounted.*

Possible epidemic curve of inhalational anthrax from a catastrophic bioterrorist attack assuming the distribution of cases observed in the 1979 Sverdlovsk anthrax epidemic



We might observe the following events:[22]

- Nothing happens in the first two days, allowing the terrorists to escape.
- On days 3 and 4, respectively, 100 and 500 people come to doctors and hospitals all over the city with fever, chest pain, flu-like symptoms and occasional chest X-rays showing only mediastinal widening, but the correct diagnosis is not suspected.

- On day 5, 1,000 new cases come in, the first 50 deaths occur, and blood cultures from the first cases prove anthrax. The press announces the epidemic, and panic ensues.
- On day 6, 1,800 new cases present, 700 deaths occur, there is no room in hospital ICUs, regular wards or hallways left anywhere in the county, and dying people are sent home or to other cities. A run on doctors' offices and antibiotics begins.
- On day 7, 2,800 new cases and 1,000 deaths occur, no antibiotics are left in North Texas, body disposal is becoming a logistical problem, bulldozers dig mass graves south of town, people leaving the city in droves, all commerce stops.
- In the second week, the epidemic peaks on the eleventh day with 4,500 new cases and 3,600 deaths, most die without medical help. By the end of the week, the total death count is estimated at 25,000.
- In the third week, the number of new cases drops each day from 3,800 per day on Monday to 500 per day by Friday. Total death toll rises to 35,000.
- In weeks 4-7 the numbers of new cases continue at about 200-500 per day. By now doctors have learned to prescribe antibiotics for the least cough or fever and the case-fatality ratio decreases from 100% to 50%.
- After the seventh week, new cases trail off rapidly, and plans for environmental cleanup are discussed.

It must be emphasized that this is a worst-case scenario that is unlikely to occur. Even though it is unlikely, however, public health planning must allow for severe illness on this scale.

Steps in a Public Health Response

In the event of a catastrophic anthrax exposure, we are currently prepared to mount an effective public health response that would considerably reduce the numbers of symptomatic infections and deaths. The key to designing a public health response to an anthrax attack is the recognition that following the initial premonitory cases, there would be a window of opportunity of several days to identify the infective agent, determine antibiotic susceptibilities, identify the group(s) at highest risk, distribute appropriate antibiotics, and develop a plan for distributing the critically ill to other locales. Delay in these steps would reduce the numbers of casualties prevented. The following are the most critical steps in such a response.

Early Recognition. The earliest opportunity to recognize the problem would be in the offices of private physicians and emergency rooms. The premonitory flu-like illness would be recognized if excessive numbers began presenting to physicians with it or if excessive school or business absenteeism were recognized. Local school districts monitor absenteeism daily and have been briefed on the need to report unusual occurrences to the health department. If an increase in flu-like illnesses were overlooked, a couple of days later an increase in critically ill patients would quickly fill the intensive care units of the local hospitals, and this would be recognized by the supervisors of Emergency Medical Services ambulance system, who would report it to the health department. Increasing awareness of the early presentation of anthrax infection among private physicians is important in minimizing the delay in early recognition.

Physicians suspecting a case of anthrax could receive consultation or activate the local public health response by calling the Dallas County Health Department (214-818-2000 or 2004), the Dallas County Medical Society (214-948-3622), the Texas Department of Health (1-800-252-8239), or the CDC 24-hour emergency notification system (770-4888-7100).

Microbiologic Diagnosis. Early identification of the anthrax organism would require physicians with a high index of suspicion to take a nasal swab for culture in patients presenting with the flu-like illness, or sending sputum, pleural fluid, cerebrospinal fluid or peritoneal aspirate for gram stain and culture in the initial critically ill patients. It should be emphasized that gram stain of clinical specimens or examination of a peripheral blood smear is likely to give the most rapid diagnosis. Anthrax grows rapidly in culture and should be isolated within 24 hours. Antimicrobial susceptibility testing should be available in 48 hours. As soon as anthrax was presumptively identified, assistance would be requested from the state health department and CDC reference laboratories.

Epidemiologic Investigation. As soon as an anthrax cluster were suspected, local public health officials, joined by state health department and CDC staff as soon as they were available, would organize an epidemiologic investigation to take histories from the initial patients. The objective would be to triangulate where the victims' path crossed or what exposures they might have had in common. If successful, this information would define a relatively small subset of the area population at high risk for having been exposed. Nasal swab culture surveys would be performed to confirm the exposure of the high risk group and lack of exposure in other groups.

Delivery of Antibiotics to the High Risk Group. If a high risk group could be identified, local news media would broadcast the parameters of the high risk group and give its members instructions on where to report to obtain examinations and prophylactic antibiotic treatment. This would be carried out with the help of police and other resources of the Dallas Office of Emergency Preparedness. Logistical plans for such distributions have been made.

The ability to obtain sufficient quantities of antibiotics for prophylaxis and treatment would be critical in reducing mortality. The amount of antibiotics required would be greatly reduced by epidemiologically identifying the high risk group, thus avoiding having to provide 60-day courses of antibiotics for the entire area population. In addition, knowing the antibiotic susceptibilities of the epidemic strain would enlarge the list of antibiotics that could be used.

In planning for sufficient antibiotic supplies, it is important to consider that the epidemic would unfold progressively over a week or more, allowing some time to bring in supplies from outside. Initially local antibiotic supplies would be used. Under the National Pharmaceutical Stockpile program, administered by CDC, antibiotics and other supplies are currently being stored in eight (soon to be 12) warehouses located in secret locations throughout the country. These supplies could be delivered to local communities within 12 hours. In addition, Congress has appropriated funds under the program for pharmaceutical companies to increase inventories so that larger quantities of antibiotics could be delivered in 48 hours. Consequently, adequate antibiotics will be available.

Intensive Care of Critically Ill Patients. In a small epidemic of inhalation anthrax, critically ill patients would be hospitalized in the ICUs of local hospitals, since intensive care may reduce the mortality rate. In a large epidemic, local ICU resources would be rapidly overwhelmed, and critically ill victims would be transported to hospitals in other cities or cared for in makeshift ICU facilities.

Final Thought

However bad an anthrax attack seems, a bioterrorist attack with smallpox, plague or botulinum toxin could be far worse! Consequently, all physicians should study the manifestations of these, and other possible, agents[1,3,4,23-26] and be prepared to make the early diagnosis and participate productively in the public health response.

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Additional Websites :

Centers for Disease Control and Prevention Bioterrorism Site: www.bt.cdc.gov (use their search engine to locate sources on "bioterrorism" and "anthrax.")

Johns Hopkins University Center for Civilian Biodefense Studies: www.hopkins-biodefense.org

APIC Bioterrorism resources (including a readiness plan template for health facilities): www.apic.org/bioterror/

US Army NBC Site (including the USAMRIID Medical Management of Biological Casualties handbook): www.nbc-med.org