

Bioterrorism and Biological Agents Part 1

Notes to accompany slides

Biologic Agents

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When Robert Koch developed his now famous Koch's Postulates, the organism that he used was *Bacillus anthracis*, an encapsulated, nonmotile midpolar spore former. The term anthracis comes from the Greek word anthrakis, which means coal, because of the distinct coal black appearance of the skin lesion after necrosis forms. The Anthrax spore is resistant to temperature extremes, some disinfectants and adverse weather conditions and can persist in soil for decades. It can be weaponized. It is believed that 10 countries, in addition to terrorist groups, have anthrax as a biologic weapon. Iraq admitted that it was weaponizing this agent prior to the Gulf War.¹ The Soviet Union had produced this agent in megaton quantities and weaponized it. Their research caused the agent to be genetically modified to be resistant to vaccines and antibiotics. The United States in the 1950s and 60s also weaponized it. In 1969 the United States policy was to discontinue the development and production of chemical and biologic weapons but rather develop research to combat their threat. Terrorists groups have used anthrax prior to September 11th. One terrorist group, Aum Shinrikyo, responsible for the release of sarin in a Tokyo, Japan, subway station in 1995, dispersed aerosols of anthrax and botulism throughout Tokyo on at least 8 occasions. For unclear reasons, the attacks failed to produce illness.

The accidental aerosolized release of anthrax spores from a military microbiology facility in Sverdlovsk in the former Soviet Union in 1979 resulted in at least 79 cases of anthrax infection and 68 deaths and demonstrated the lethal potential of anthrax aerosols. It also indicated that the Soviet Union did not live up to its agreement to discontinue production of these types of agents.

An anthrax aerosol, properly weaponized, would be odorless and invisible following release and would have the potential to travel many kilometers before disseminating. Evidence suggests that following an outdoor aerosol release, persons indoors could be exposed to a similar threat as those outdoors.²

A 1993 report by the US Congressional Office of Technology Assessment estimated that between 130,000 and 3 million deaths could follow the aerosolized release of 100 kg of anthrax spores upwind of the Washington, DC, and area - lethality matching or exceeding that of a hydrogen bomb.³

In the natural state this agent tends to clump up and serves as a cutaneous pathogen entering through small cuts or abrasions. Prior to September 11th, 95% of anthrax was of the cutaneous type. The spores are about 1 u in size but the clumps of spores are not of respirable size to enter the deep airways. Some workers normally breathe 400 spores of anthrax daily in their occupation and do not develop an illness.

Advanced preparation methods can "mill" this agent in a respirable size. This is what has occurred in the recent terrorist attacks. Anthrax is not known to cause person to person transmission.⁴

1. Zilinskas RA. Iraq's biological weapons: the past as future?

JAMA.

1997;278:418-424.

2. Meselson M, Guillemin J, Hugh-Jones M, et al.

The Sverdlovsk anthrax outbreak of 1979.

Science.

1994;266:1202-1208.

3. Office of Technology Assessment, US Congress.

Proliferation of Weapons of Mass Destruction.

Washington, DC: US Government Printing Office; 1993:53-55. Publication OTA-ISC-559.

4. Anthrax as a biologic weapon: A consensus statement JAMA Vol. 281 No. 18,
May 12, 1999

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Note the typical “boxcar” like appearance of this bacillus on this scanning electron microphotograph. This organism grows easily on standard culture media producing an organism showing a typical jointed bamboo appearance. The colonies form a characteristic “curled hair” appearance. It does not produce hemolysis on sheep blood agar plates. If specifically looked for, the appearance on culture and slides are very accurate in identifying this organism. Another bacillus appearing similarly in this country, and found much more frequently, is the relatively harmless *Bacillus cereus*. The culture only takes 6 to 24 hours to grow the organism at 37°. Besides a culture, more specialized testing including immunofluorescence, immunodiffusion, enzyme-linked immunosorbent assay for protective antigen, polymerase chain reaction, and specific antibodies to anthrax are available at the U.S. Army Medical Research Institute for Infectious Disease (USAMRIID) or the Centers of Disease Control and Prevention for confirmation of the disorder and better characterization of the strain. The local or state health department must be contacted early in cases of confirmed or suspect anthrax as they are either able to confirm the presence of anthrax or coordinate with the sophisticated laboratory network organized specifically for the threat of bioterrorism (LAN) to rapidly get this information confirmed.

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Notice the midpole location of the spores. Spores form when exposed to an oxygen rich environment. They are not found in a human unless there is an open wound that exposes the vegetative stage to oxygen.

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Robert Koch's original slides showing *Bacillus anthracis*.

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The gastrointestinal form has a 60% mortality if untreated and occurs from ingestion of contaminated meat. Nausea, vomiting, diarrhea and gastrointestinal bleeding occur. The terminal ileum is most affected. Esophageal ulcerations and other loci of bleeding occur. The high mortality rate is due to the difficulty in making the diagnosis, especially in third world countries where these conditions are seen. Another form of the gastrointestinal type from eating partially or uncooked infected meat is the oropharyngeal form. It has not been reported in the United States.

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Most cutaneous anthrax occurs on the extremities or the face.

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Cutaneous anthrax - A small pruritic papule begins initially followed in some cases by a vesicle or ulcer, then an area of central necrosis. The necrotic lesion of anthrax may or may not ulcerate. Aspirate from the vesicle is a good source for culturing the agent. There is often persistent surrounding erythema and/or edema. If untreated, this form of the disease has a 20% mortality. It must be differentiated from other ulcerative focalized lesions such as a spider bite, glanders, tularemia. Anthrax is sensitive to many antibiotics in the native state including erythromycin, quinolones of all types, penicillin and tetracyclines. It is resistant to cephalosporins. There are about 2000 cases of cutaneous anthrax reported per year in the United States, most in individuals handling animal hides. Systemic effects such as fatigue, headaches, fever and myalgia may be seen with this. The seven month old confirmed anthrax case had hemolytic anemia and severe thrombocytopenia affiliated with it.

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Inhalational anthrax - Between 1900 and 1978 only 18 cases of inhalational anthrax were reported. Two were in laboratory workers, most of the others in goat hair mill and goatskin workers, wool workers and tanners. In fact another name for inhalation anthrax is wool sorter's disease.

Inhalational anthrax is usually an acute infectious disease characterized by non-specific flu like symptoms with fever, chills, vomiting, cough, headache, chest and abdominal pain and weakness for several days and then a period of apparent improvement in some but not all the cases, lasting hours to days, followed by the second phase of sudden dyspnea, diaphoresis, shock, cyanosis and rapid death in hours to a few days. Based on primate data, it has been estimated that

for humans the LD 50 (lethal dose sufficient to kill 50% of persons exposed to it) is a 2500 to 55,000 inhaled anthrax spore.

In this file a widened mediastinum with a pleural effusion is seen. Note that a hemorrhagic mediastinitis and lymphangitis with necrosis are seen in these cases. No true pneumonia is involved. 50% of the cases develop meningitis with evidence of mental obtundation, delirium and meningismus.

In this form of the disease, the bacteria spread to lymphoid tissue where they multiply rapidly, with a doubling time of about 25 minutes. Once the hematogenous and lymphatic spread to the mediastinal lymph nodes occur, these organisms produce toxins known as edema factor, lethal factor and protection antigen. In addition these organisms produce an anti-phagocytic capsule. They also institute a massive activation of cytokines. This combination accounts for a rapid shock-like state and death following a non-specific onset. Germination of the spores in some cases may take an extended period of time to manifest illness. In Sverdlovsk germination from the spore to the vegetative state occurred from 2 to 43 days after exposure. Once the toxins are produced, death occurs in experimental animals even if all the anthrax is killed with antibiotics.

The anthrax vaccine produced by BioPort Laboratories in Michigan is 93% effective. The vaccine has been used since 1976 but is currently not being manufactured as the plant is undergoing renovation. Resumption of production is expected soon. It is not currently available for the civilian population except for rare exceptions. Anthrax vaccinations require a series of six injections over an 18-month period, followed by one booster injection once a year. It would be difficult, but not impossible, for a massive civilian vaccination to occur.

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This is mediastinal medullary necrosis. Diffuse perivascular infiltration is also present. A sputum analysis and gram stain is unlikely to be reliable given a lack of true bronchopneumonia.

In the inhalational form, a widened mediastinum on x-ray in a previously healthy individual initially presenting with flu-like symptoms is almost pathognomonic for inhalational anthrax. Anthrax is characterized by flu-like symptoms that appear to get much better for hours to days. Increasing dyspnea, collapse and death in 1 to 3 days then follows this. The high mortality rate with the inhalation form was based on earlier case reports without the intensive medical support currently available. Antibiotics such as 400 mg Ciprofloxacin IV BID may improve survival, especially if caught early. Other variables that influence survival include underlying diseases, age, immune status, nutritional status, tobacco use and genetic variability. There is a higher probability of inhalational anthrax with weaponized anthrax, which is defined as modification of the organism to act to purposely cause disease in man. It can be done by modifying the genetics of the organism to make it more resistant to antibiotics or vaccines or to eliminate the clumping tendency by sophisticated methods to permit micro dispersion into the lower airways.

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The above slide represents the recommendations to prevent inhalational anthrax in those known to be exposed according to the CDC. This information is available in the MMWR (Oct 19 2001 50:40 893). The CDC also recommends that children who are not allergic to penicillin should be switched to amoxicillin 80mg/kg/day divided to be taken every 8 hours if the organism is confirmed to be sensitive to penicillin. Domestic unaltered anthrax would be expected to be penicillin sensitive. Note that ciprofloxacin was recommended by the CDC for a very important reason. Bioengineered anthrax in the former Soviet Union was specifically altered to be resistant to penicillin and tetracycline. The soviet state ceased and supposedly the manufacturing of biologic weapons ceased in 1992 when Yelsin informed the world that the Soviet union had not complied with various treaties banning biological and chemical agents starting with the 1927 Geneva Convention. If taken too late, antibiotics have had a limited efficacy in the inhalation form, reducing the death rate from 97 to 88%. With more awareness and earlier antibiotic administration, it is hoped that the survival rate will be much better since our statistics are based on a limited amount of cases many years ago.

Once suspected or known, the inhaled form requires the intravenous administration of 400mg of ciprofloxacin every 12 hours or Doxycycline 100 mg IV every 12 hours. One of these agents should be combined with rifampin, chloramphenicol, imipenem, clindamycin, vancomycin, amoxicillin, clavulanic acid or clarithromycin. In addition supportive care can be critical. Surgery and autopsies raise the specter of spores. Systemic symptoms may occur with the cutaneous form and dual or even triple antibiotic therapy is being advocated in such cases.

There are no easy answers to some of these scenarios though it is recommended to obtain blood, CSF and mediastinal aspirate in lieu of an autopsy to confirm the disease. Sterilizing the environment is possible using concentrated formaldehyde, glutaraldehyde or EtO. Currently chloride

dioxide is being used followed by bisulfide. It becomes more difficult in dealing with the home environment.

New approaches to the treatment of anthrax on the horizon - Efforts are currently being made to use a more pure anthrax vaccine to provide protection more safely. Also the cellular receptor that anthrax toxin attaches to has been identified and copied. The Lethal factor has been crystallized and duplicated. Peptides have been produced that experimentally have prevented the mouse from dying of anthrax. Manipulating receptors and toxic interactions may well be valuable tools for future therapy.

1. Bradley, K.A., Mogridge, J., Mourez, M., Collier, R.J. & Young, J.A.T. Identification of the cellular receptor for the anthrax toxin. Nature, (2001).
2. Pannitter, A. et al. Crystal structure of the anthrax lethal factor. Nature, (2001).
3. Watters, J.W. et al. Kif1C, a kinesin-like motor protein, mediates more macrophages resistance to anthrax lethal factor. Current Biology, 11, 1503 - 1511, (2001).
4. Mourez, M. et al. Designing a polyvalent inhibitor of anthrax toxin. Nature Biotechnology, 19, 958 - 961, (2001).
- 5.5.

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If there is an alleged or suspected case of anthrax, contact law enforcement to try to substantiate the validity of the allegation in order to avoid unnecessary testing and treatment.

The CDC defines a confirmed case of anthrax as 1) a clinically compatible case of cutaneous, inhalational, or gastrointestinal illness* that is laboratory confirmed by isolation of *B. anthracis* from an affected tissue or site or 2) other laboratory evidence of *B. anthracis* infection based on at least two supportive laboratory tests. A suspected case was defined as 1) a clinically compatible case of illness without isolation of *B. anthracis* and no alternative diagnosis, but with laboratory evidence of *B. anthracis* by one supportive laboratory test or 2) a clinically compatible case of anthrax epidemiologically linked to a confirmed environmental exposure, but without corroborative laboratory evidence of *B. anthracis* infection.

Laboratory criteria for diagnosis of anthrax consist of 1) isolation and confirmation of *B. anthracis* from a clinical specimen collected from an affected tissue or site or 2) other supportive laboratory tests, including (a) evidence of *B. anthracis* DNA by polymerase chain reaction (PCR) from specimens collected from an affected tissue or site, (b) demonstration of *B. anthracis* in a clinical specimen by immunohistochemical staining, or (c) other laboratory tests (e.g., serology) that may become validated by laboratory confirmation.

Remember terrorism is a war on two fronts. You, the physician, are a soldier in the front lines on the home front. Be knowledgeable and compassionate and keep up with the latest information at www.bt.cdc.gov.

Do not look at the health department as you once may have. They are your right hand in the fight for a safer world. New trust and teamwork with the state and local health department is now essential. Keep their number close at hand.

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Ground Zero

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Smallpox is a member of the Orthopoxviridae family. It is a large encapsulated DNA virus. There are 187 putative proteins that have been sequenced from the Variola virus. 150 of these are the same as vaccinia virus.

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Boy with smallpox from the CDC galleries. The lesions are found heavily on the face and also the palms and soles. This is much less likely to be seen in varicella cases.

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Vaccinia can occasionally occur. A times local major reactions such as the one shown on the above slide occurs. Those of us who are older and took smallpox vaccinations many years ago, no longer have immunity. It is recommended that if you are immunized in the future, you get another booster treatment every 10 yrs to maintain the immunity.

The CDC published a report in 1968 that describes the complications of vaccinia (smallpox) vaccine. Early efforts to use smallpox from scars cause serious problems in about 1 out of 200 cases so it was decided to use another pox virus, vaccinia, related to cowpox to create the vaccine since it is much more benign. Complications are rare but include autoinoculation and secondary inoculation. Stevens Johnson syndrome, eczema vaccinatum, progressive vaccinia and post vaccinia encephalitis. Vaccinia - Immune Globulin (VIG) is of value in treating these complications. Topical idoxuridine can be helpful in treating vaccinia keratitis. In the future do not vaccinate those with HIV or serious immune deficiency disorders, those with eczema and certain other precautions which should be reviewed at the CDC website if the vaccine again becomes available.

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Note the progression from pustules to scabs to scars.

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Throughout the time of the eruption, typically the fever remains. The skin changes are painful. There is a 30% death rate, which typically occurs during the second week of the disease. All skin lesions appearing in this disease appear at the same stage of development. This differs from chicken pox in that crops of lesions at different stages of development occur. Chicken pox skin lesions are less on the face and extremities and are denser on the trunk. The opposite is true with smallpox. Smallpox is infectious by aerosol droplets from the initiation of the rash. It remains infectious until the last of the scabs drops off.

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Strict respiratory quarantine for 17 days from all contacts with the index case is required. The United States currently has enough doses of Smallpox vaccine to inoculate 77 million people. The World Health Organization has enough to inoculate another 200 million. Currently there is an effect to see if our existing stockpiles, if diluted, can still confer immunity. If so, this will triple the amount available. The United States entered an agreement with a manufacturer (OraVax) to produce more vaccine but it will take time to get into production. It will be a live vaccinia vaccine like the current vaccine and delivered with a bifurcated needle (modified scarification method).

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Bocklin's depiction of the Black Death

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Yersinia Pestis, the cause of plague. This bacteria is a nonmotile aerobic gram-negative rod of the family Enterobacteraceae along with Yersinia enterocolitica and Y. pseudotuberculosis. Plague is a zoonotic disease of rodents such as ground squirrels, mice, and rats. At times birds, rabbits or cats can be infected. Man is a secondary host. Often large amounts of rats will die first during a plague epidemic and then the flea, Xenophyllus cheopsis, will infect man.

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The flea behind it all, Xenophyllus cheopsis.

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Buboes were named after boubon, a swollen, infected lymph node – Latin for groin. In the Middle ages several well-known epidemics of the “Black plague” occurred killing one third of the population of Europe and England. Since the epidemic of 1347 started in China we have no way to know the amount of death caused there but it is estimated to be 13 million. Every winter for 5 years it would get better since the fleas became dormant. every spring it would return so that 25 million deaths occurred over a 5 year period. Gradually it became less until it died out in the 16th Century.

In the bubonic form, is the most common form and though found rarely in the United states is most likely found in northern Arizona, northern New Mexico or southern Colorado, Septicemia is common in bubonic plague since 80% of blood cultures are positive. Only about one quarter of the cases develop into the clinical septicemic stage. Along the course of the lymphatics emanating from the bubo, papules or pustules can sometimes be seen. Material from the infected bubo will usually be positive. The mortality rate of an untreated bubonic plague patient is 60%.this can be reduced to

5 to 10% with antibiotics. Rarely the septicemic form of plague will develop initially so that it resembles the clinical picture of bubonic plague but without any buboes.

Tetracyclines, quinolones, chloramphenicol, and Gentamycin are all effective treatments. The CDC in fact recommends sulfonamides in children less than 8.

With secondary or clinical septicemia gram negative endotoxemia develops with chills, diarrhea, hypotension, vomiting and fever. In this stage, hemorrhage under the skin, thrombosis of acral vessels, disseminated intravascular coagulation, gangrene of the appendages and dark purpura proximally are seen.

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Plague pneumonia with large amounts of *Y. pestis* seen. Plague pneumonia can occur as a primary event or secondary to septicemia from a bubonic infection. Buboes are rare with this form but can be present. X-rays show bilateral infiltrates, which may be patchy or consolidated.

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Yersinia may be missed if the lab is not directly informed to look for it. It grows well on MacConkey or blood agar. The colony appearance is atypical for a gram negative organism and can be an important clue.

The pneumonic form is very contagious. Contact with such a person requires the prophylactic administration of tetracyclines. There is a 6% plague meningitis rate.

Diagnosis is made by finding gram negative organism with Gram, Giemsa, Wright or Wayson's stained specimens of the sputum, blood, spinal fluid or lymph node aspirate. F1 antigen immunoassays or PCR test for the organisms are helpful and confirmatory but there is no substitute for clinical suspicion in this disease since there is not enough time to wait for blood tests results in most cases.

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Though DIC is present, it rarely requires heparin, hypotension responds to crystalloid replacement and at times pressor agents.

A plague vaccine has some measure of protection against the bubonic form of the disease but none against the aerosol form. This vaccine is in limited supply at the CDC and the manufacturer recently stopped production.

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Medical robe of the middle ages for protecting oneself from plague. The long robe was covered with wax so that the miasma would be deflected from the physician. The beaklike wooden nozzle was filled with perfume type material. One critic states that he believes that it doesn't work well but does tend to keep away the fleas. They almost got it right.

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Stevan Cordas DO MPH