How To Weaponize Anthrax?

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Anthrax, a zoonotic disease caused by *Bacillus anthracis*, occurs in domesticated and wild animals-primarily herbivores. Humans usually become infected by contact with infected animals or their products. Anthrax is so easy to obtain that it could be weaponized for biological warfare if a laboratory area of 5 m² is owned with 10.000$.

Key words: *Anthrax, weapon, spore,* Bacillus anthracis

Bacillus anthracis (B. anthracis), the bacterium that causes anthrax, is one of the most serious of the biological agents that may be used as weapons. Anthrax occurs in domesticated and wild animals-primarily herbivores including goats, sheeps, cattles, horses and swines. Humans usually become infected by contact with infected animals or contaminated animal products. Infection occurs most commonly via cutaneous route and only very rarely via respiratory or gastrointestinal routes (1). Anthrax is easy to obtain. Effective aerosolizing devices made for commercial use can be purchased from electronics or home improvement stores (2). From the military point of view, *B. anthracis* is the most desirable of the biological agents to be used in warfare due to the reasons given in Table I.

Bacillus anthracis:

B. anthracis is an aerobic, gram-positive, spore-forming, nonmotile *Bacillus* species. The vegetative cell size is 1-1.5 x 1-8 μm and spore size is aproximately 1 μm that grows readily on conventional microbiology media, including blood agar. *B. anthracis* spores germinate when they enter an environment rich of amino acids, nucleosides, and glucose, similar to that of found in the blood or tissues of animal or human host. *B. anthracis* forms a large polypeptide capsule detectable in clinical specimens (3) (Fig. 1).

The anthrax toxin comprises three proteins. One anthrax protein, called protective antigen (PA) chaperones, the two other toxins, which can enter into human or animal cells and shield them from the body's immune system. The second, lethal factor (LF), destroys the white blood cells that function in immune defence. LF, is a zinc metalloprotease that inactivates mitogen-activated protein kinase inducing cell death. The third toxin molecule, edema factor (EF), hijacks the signaling system in the organism. EF is a Ca+/calmodulin-dependent adenylyl cyclase impairing host defences (4). This disrupts the energy balance of cells and leads to the accumulation of fluid and complete destruction of the cells (5). Each of three proteins exhibits no significant biological activity in animals. However, combinations of two or three of the toxin components yield the following results in experimental animals.

PA+LF combine to produce lethal activity, EF+PA produce edema, EF+LF is inactive, PA+LF+EF produces edema and necrosis and is lethal (4).

The virulence of *B. anthracis* is attributable to four bacterial components (6):

1. Capsular material composed of poly-D-glutamate polypeptide,
2. EF component of exotoxin,
3. LF component of exotoxin,
4. PA component of exotoxin.

Historical aspect of use of anthrax weapon:

Research on anthrax as a biological weapon began more than 80 years ago (7). At least 13 nations are believed to have offensive biological weapons programs. In 1995, Iraq has acknowledged producing and weaponizing *B. anthracis* to the United Nations Special Commission (8). 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. 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 (9).

In September 2001, *B. anthracis* spores were sent to several locations via the US Postal Service. Twenty-two confirmed or suspected cases of anthrax infection resulted. Eleven of these were inhalational cases, of whom 5 died; 11 were cutaneous cases (8) (Table II).

Making weapons-grade anthrax;

Anthrax is the mostly preferred biological warfare agent because:

It is highly lethal. A hundred million lethal doses per gram of anthrax material will be adequate (100,000 times deadlier than any deadliest chemical warfare agent).

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Table I. The advantages of *Bacillus anthracis* and endospores as a weapon.

- It is easy to grow,
- It forms spores which makes it an agent easy to store, resistant to sunlight and heat,
- It will only infect those directly exposed,
- There is a vaccine for the deployable troops (if available),
- Antibiotic therapy may not work after the first symptoms appear.

Table II. How to handle suspicious packages and letters?

- Do not shake or empty the contents of any suspicious envelope or package.
- Place the envelope or package in a plastic bag or some other type of container to prevent leakage of contents.
- If you do not have any container, then cover the envelope or package with anything (e.g., clothing, paper, trash can, etc.) and do not remove this cover.
- Do not try to clean up the powder.
- Turn off local fans or ventilation units in the room.
- Then leave the room and close the door, or section off the area to prevent others from entering (i.e., keep others away).
- Wash your hands with soap and water to prevent spreading any powder to your face.

Table III. Recommendations for medical therapy for patients with clinically evident inhalational anthrax infection in the contained casualty setting.

	Initial therapy	Optimal therapy if strain is proven susceptible	Duration (day)
Adults	Ciprofloxacin, 400 mg intravenously every 12 h	Penicillin G, 4 million U intravenously every 4 h Doxycycline, 100 mg intravenously every 12 h	60
Children	Ciprofloxacin, 20-30 mg/kg per day intravenously divided into 2 daily doses, not to exceed 1 g/d	Age ,12 y: penicillin G, 50 000 U/kg intravenously every 6 h Age 12 y: penicillin G, 4 million U intravenously every 4 h	60
Pregnant women	Same as for nonpregnant adults		
Immunosuppressed persons	Same as for nonimmunosuppressed adults and children		

Inhalational anthrax is virtually always fatal.

There are low barriers for production. Low cost production of the anthrax material does not require a high-technology. Knowledge is widely available for easy production in large quantities.

It is easy to be weaponized. It is extremely stable and can be stored almost indefinitely as a dry powder. It can be loaded, in a freeze-dried condition, in munitions or disseminated as an aerosol with crude sprayers.

Given appropriate weather and wind conditions, 50 kilograms of anthrax released from an aircraft along a 2-kilometers line could create a lethal cloud of anthrax spores that would extend beyond 20 kilometers downwind. The aerosol cloud would be colorless, odorless and invisible following its release. Given the small size of the spores, people indoors would receive the same amount of exposure as people on the street. A bioterrorist attack would carry an economic burden of $26.2 billion per 100,000 people exposed to the spores (9).

Inhaled infectious dose is estimated to be 4.000-80.000 spores (10). Owing to the infectivity of anthrax spores by the respiratory route and the high mortality of inhalation anthrax, the military's concern with anthrax is with its potential use as a biological warfare agent. The disease begins with nonspecific symptoms followed in 2 to 3 days by the sudden onset of respiratory distress with dyspnea, cyanosis and stridor. It is rapidly fatal. Radiographic examination of the chest often reveals the characteristic mediastinal widening, indicative of hemorrhagic mediastinitis. Hemorrhagic meningititis frequently coexists. To cause inhalation anthrax, the particle size needs to be between 1-5 μm to reach the lower lung and

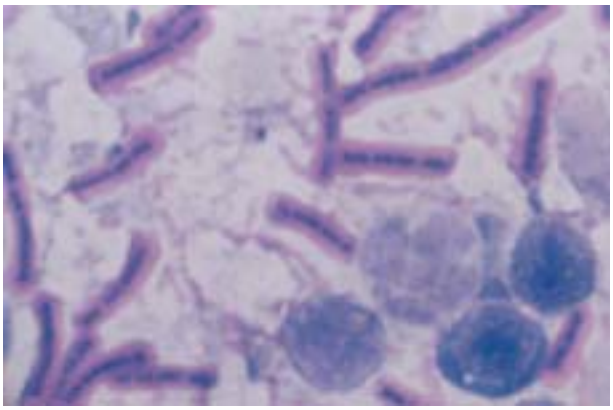

Figure 1. *Bacillus anthracis*-Gram stained. The cells have characteristic squared ends. *B. anthracis* forms a large polypeptide capsule in clinical specimens.

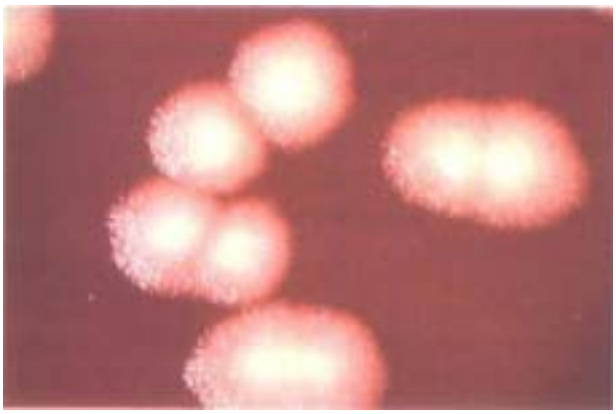

Figure 2. Several nonselective and selective media for the detection and isolation of *Bacillus anthracis* have been described, as well as a rapid screening test for the bacterium based on the morphology of microcolonies.

cause infection. Anything larger than this will stick in the upper respiratory tract where a much higher dose would be needed to cause disease. Given the rarity of the diseases and its rapid progression, the diagnosis of inhalational anthrax is difficult to make (10).

There is no evidence of person-to-person transmission of anthrax. Quarantine of affected individuals is not recommended. Anthrax spores may survive in the soil, water and on surfaces for many years. Spores can only be destroyed by steam sterilization or burning (2).

Anthrax culture

Cultures should be processed and examined with great care in a biologic safety cabinet (3). Every precaution should be taken to avoid the production of aerosols of the infected material. Laboratory personnel should wear protective coats or gowns, masks, and surgical gloves when processing the samples. This safety apparel should be autoclaved before it is reused or should be discarded. When the work is finished, all surface in the biologic safety cabinet and laboratory workbenches must be decontaminated with 5% hypochlorite solution, and all instruments used for processing and the specimen must be autoclaved (10).

B. anthracis grows well on ordinary blood agar within 18 to 24 hours at 35 °C. Typically the colonies are flat and irregular, are 4 to 5 mm in diameter, and have a slightly undulate margin when grown on heart infusion blood agar. The organism is not hemolytic on sheep blood agar, which is a helpful in differentiating *B. anthracis* from alpha and beta-hemolytic isolates of other *Bacillus* species. Under the dissecting microscope, numerous ondulated outgrowths consisting of long filamentous chains of bacilli may be seen (3) (Fig. 2).

Conclusion

In conclusion, anthrax is very easy to grow, harvest and store. Dispersal is not too difficult if small area or small numbers of people were to be targeted.

Specific recommendations include diagnosis of anthrax infection, indications for vaccination, therapy, postexposure prophylaxis, decontamination of the environment, and suggested research.

Standard precautions are recommended for patient care. There is no evidence of direct person-to-person spread of disease from inhalational anthrax. After an invasive procedure or autopsy, the instruments and area used should be thoroughly disinfected with a sporicidal agent. Chlorine, in the form of sodium or calcium hypochlorite, can be used, but with the caution that the activity of hypochlorites is greatly reduced in the presence of organic material.

The anthrax vaccine (anthrax vaccine absorbed [AVA]) has been licensed by the FDA since 1970 for the preexposure prophylaxis for inhalational anthrax in persons at risk of acquiring the disease occupationally, such as woolen mill workers, laboratory workers, and veterinarians. The vaccine provides almost 100% protection to nonhuman primates against an aerosol challenge with *B. anthracis*. There are no FDA-approved postexposure antibiotic regimens following exposure to an anthrax aerosol. For postexposure prophylaxis, the working group recommends the same antibiotic regimen as that recommended for treatment of mass casualties; prophylaxis should be continued for 60 days (Table III) (2, 8,9).

References

1. Lew D: Bacillus anthracis (anthrax) in: Principles and Practices of Infectious Disease. Eds. Mandell GL, Bennett JE, Dolin R., New York, NY: Churchill Livingstone Inc 1995, pp: 1885-1889.
2. Kortepeter M, Chritopher C, Cieslak T,Culpepper R: Medical Management of Biological Casualties Handbook, 4th ed, Fort Detrick, Maryland, 2001, pp: 21-26.
3. Doyle RJ, Keller KF, Ezzel JW: Bacillus in: Manual of Clinical Microbiology, 4th ed, Lennette EH, Balows AW (eds), Washington DC, American Society for Microbiology, 1985, pp: 211-215.
4. Ascenzi P, Visca P, Ippolito G, Spallarossa A, Bolognesi M, Montecucco C: Anthrax toxin: a tripartite lethal combination. FEBS Lett, 531: 384-388, 2002.

5. Kralove H: Anthrax toxin characterization. Acta Medica; 45: 3-5, 2002.
6. Smith H: Discovery of the anthrax toxin: the beginning of studies of virulence determinants regulated in vivo. Int J Med Microbiol 291: 411-417, 2002.
7. Christopher GW, Cieslak TJ, Pavlin JA, Eitzen EM: Biological warfare: a historical perspective. JAMA 278: 412-417, 1997.
8. Inglesby TV, O'Toole T, Henderson DA, Bartlett JG: Anthrax as a biological weapon. JAMA 287: 2236-2252, 2002.
9. Inglesby TV, Henderson DA, Bartlett JG: Anthrax as a biological weapon. JAMA 281: 1735-1745, 1999.
10. Feeley JC, Patton CM. Bacillus in Manual of Clinical Microbiology, 3rd ed, Lennette EH, Balows AW (eds), American Society for Microbiology, Washington DC, 1980, pp: 145-149.

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