

Bacillus

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General Concepts

Clinical Manifestations

Anthrax is caused by *Bacillus anthracis*. Humans acquire the disease directly from contact with infected herbivores or indirectly via their products. The clinical forms include (1) cutaneous anthrax (eschar with edema), from handling infected material (this accounts for more than 95 percent of cases); (2) intestinal anthrax, from eating infected meat; and (3) pulmonary anthrax, from inhaling spore-laden dust. Several other *Bacillus* spp, in particular *B cereus* and to a lesser extent *B subtilis* and *B licheniformis*, are periodically associated with bacteremia/septicemia, endocarditis, meningitis, and infections of wounds, the ears, eyes, respiratory tract, urinary tract, and gastrointestinal tract. *Bacillus cereus* causes two distinct food poisoning syndromes: a rapid-onset emetic syndrome characterized by nausea and vomiting, and a slower-onset diarrheal syndrome.

Structure and Classification

Bacillus species are rod-shaped, endospore-forming aerobic or facultatively anaerobic, Gram-positive bacteria; in some species cultures may turn Gram-negative with age. The many species of the genus exhibit a wide range of physiologic abilities that allow them to live in every natural environment. Only one endospore is formed per cell. The spores are resistant to heat, cold, radiation, desiccation, and disinfectants. *Bacillus anthracis* needs oxygen to sporulate; this constraint has important consequences for epidemiology and control. *In vivo*, *B anthracis* produces a polypeptide (polyglutamic acid) capsule that protects it from phagocytosis. The genera *Bacillus* and *Clostridium* constitute the family *Bacillaceae*. Species are identified by using morphologic and biochemical criteria.

Pathogenesis

The virulence factors of *B anthracis* are its capsule and three-component toxin, both encoded on plasmids. *Bacillus cereus* produces numerous enzymes and aggressins. The principal virulence factors are a necrotizing enterotoxin and a potent hemolysin (cereolysin). Emetic food poisoning probably results from the release of emetic factors from specific foods by bacterial enzymes.

Host Defenses

The reasons for marked differences in susceptibility to anthrax among different animal species are not known. The protective actions of the live-spore animal vaccine or the human chemical vaccines are based on induction of humoral and cell-mediated immunity to the protective antigen component of anthrax toxin.

Epidemiology

Individuals at risk for anthrax include those in contact with infected animals or animal products. Episodes of *B cereus* food poisoning occur sporadically worldwide and result from ingestion of contaminated food in which the bacteria have multiplied to high levels under conditions of improper storage after cooking.

Diagnosis

Cutaneous anthrax is diagnosed on the basis of the characteristic papule (early) or eschar (later) with extensive surrounding edema, backed by a history of exposure to animals or their products. Diagnosis is confirmed by observation of characteristic encapsulated bacilli in polychrome methylene blue-stained smears of blood, exudate, lymph, cerebrospinal fluid, etc., and/or by culture. Other *Bacillus* infections are diagnosed by culture of the bacteria.

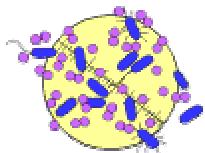
Control

Anthrax: Control in animals is essential for control in humans. In endemic areas, animals that die suddenly should be handled cautiously and livestock should be vaccinated annually. A human vaccine is available for individuals in high-risk occupations. Anthrax is readily treated with antibiotics (e.g., penicillin, tetracycline, chloramphenicol, gentamicin, or erythromycin).

Other *Bacillus* Infections: Control is by good hygiene. Treatment is with non- β -lactam antibiotics for Gram-positive bacteria. Food poisoning is controlled by adequate cooking, avoidance of recontamination of cooked food, and proper storage (efficient refrigeration).

Pharmaceutical, Agricultural, and Industrial Importance

Many of the physiologic properties and specialized metabolites of *Bacillus* species are used in the pharmaceutical, agricultural, and food industries. On the other hand, the resistance of the spores to sterilization and disinfection makes them problem contaminants in foods, medical supplies, surgical procedures, etc.



INTRODUCTION

Bacillus species are aerobic, sporulating, rod-shaped bacteria that are ubiquitous in nature. *Bacillus anthracis*, the agent of anthrax, is the only obligate *Bacillus* pathogen in vertebrates. *Bacillus larvae*, *B. lenticularis*, *B. popilliae*, *B. sphaericus*, and *B. thuringiensis* are pathogens of specific groups of insects. A number of other species, in particular *B. cereus*, are occasional pathogens of humans and livestock, but the large majority of *Bacillus* species are harmless saprophytes.

Anthrax has afflicted humans throughout recorded history. The fifth and sixth plagues of Egypt described in Exodus are widely believed to have been anthrax. The disease was featured in the writings of Virgil in 25 BC and was familiar in medieval times as the Black Bane. It was from studies on anthrax that Koch established his famous postulates in 1876, and vaccines against anthrax (the best known being that of Pasteur (1881)) were among the first bacterial vaccines developed.

Bacillus species are used in many medical, pharmaceutical, agricultural, and industrial processes that take advantage of their wide range of physiologic characteristics and their ability to produce a host of enzymes, antibiotics, and other metabolites. Bacitracin and polymyxin are two well-known antibiotics obtained from *Bacillus* species. Several species are used as standards in medical and pharmaceutical assays.

The spores of the obligate thermophile *B. stearothermophilus* are used to test heat sterilization procedures, and *B. subtilis* subsp *globigii*, which is resistant to heat, chemicals, and radiation, is widely used to validate alternative sterilization and fumigation procedures. Certain *Bacillus* species are important in the natural or artificial degradation of waste products. Some *Bacillus* insect pathogens are used as the active ingredients of insecticides.

Because the spores of many *Bacillus* species are resistant to heat, radiation, disinfectants, and desiccation, they are difficult to eliminate from medical and pharmaceutical materials and are a frequent cause of contamination. *Bacillus* species are well known in the food industries as troublesome spoilage organisms.

Clinical Manifestations

Although anthrax remains the best-known *Bacillus* disease, in recent years other *Bacillus* species have been increasingly implicated in a wide range of infections including abscesses, bacteremia/septicemia, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmritis, osteomyelitis, peritonitis, and respiratory and urinary tract infections. Most of these occur as secondary or mixed infections or in immunodeficient or otherwise immunocompromised hosts (such as alcoholics and diabetics), but a significant proportion are primary infections in otherwise healthy individuals. Some of these infections are severe or lethal. Of the species listed in Table 15-1, most frequently implicated in these types of infection is *B. cereus*, followed by *B. licheniformis* and *B. subtilis*. *Bacillus alvei*, *B. brevis*, *B. circulans*, *B. coagulans*, *B. macerans*, *B. pumilus*, *B. sphaericus*, and *B. thuringiensis* cause occasional infections. As secondary invaders, *Bacillus* species may exacerbate preexisting infections by producing either tissue-damaging toxins or metabolites such as penicillinase that interfere with treatment.

Bacillus cereus is well known as an agent of food poisoning, and a number of other *Bacillus* species, particularly *B. subtilis* and *B. licheniformis*, are also incriminated periodically in this capacity.

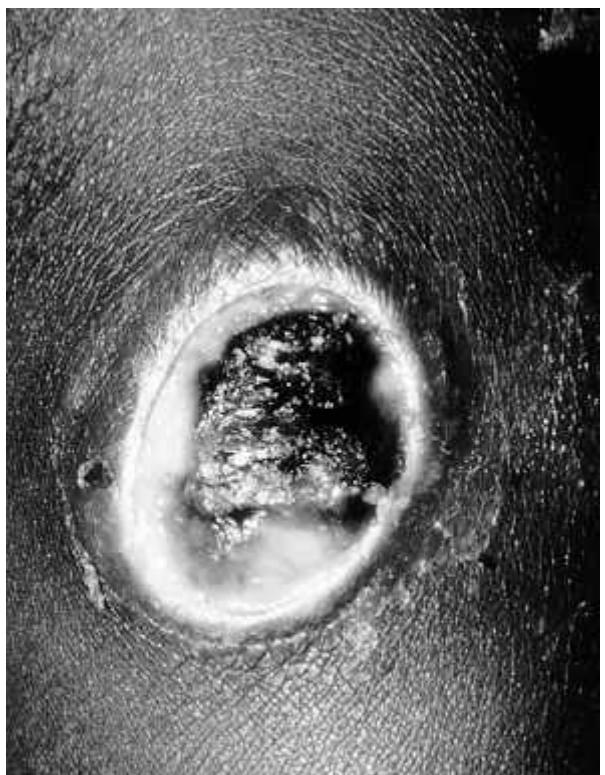
Anthrax

Anthrax is primarily a disease of herbivores. Humans acquire it as a result of contact with infected animals or animal products. In humans the disease takes one of three forms, depending on the route of infection. Cutaneous anthrax, which accounts for more than 95 percent of cases worldwide, results from infection through skin lesions; intestinal anthrax results from ingestion of spores, usually in infected meat; and pulmonary anthrax results from inhalation of spores.

Cutaneous anthrax usually occurs through contamination of a cut or abrasion, although in some countries biting flies may also transmit the disease. After a 2- to 3-day incubation period, a small pimple or papule appears at the inoculation site. A surrounding ring of vesicles develops. Over the next few days, the central papule ulcerates, dries, and blackens to form the characteristic eschar (Fig. 15-1). The lesion is painless and is surrounded by marked edema that may extend for some distance. Pus and pain appear only if the lesion becomes infected by a pyogenic organism. Similarly, marked lymphangitis and fever usually point to a secondary infection. In most cases the disease remains limited to the initial lesion and resolves spontaneously. The main dangers are that a lesion on the face or neck may swell to occlude the airway or may give rise to secondary meningitis. If host defenses fail to contain the infection, however, fulminating septicemia develops. Approximately 20 percent of untreated cases of cutaneous anthrax progress to fatal septicemia. However, *B. anthracis* is susceptible to penicillin and other common antibiotics, so effective treatment is almost always available.







D



E

FIGURE 15-1 Evolution of an anthrax eschar in a 4-year-old boy. (A&B) the lesion when first seen (day 0). Note the arm swollen from the characteristic edema. (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 kindly supplied by W.E. Kobuch, M.D., St. Luke's Hospital, Lupane, Bulawayo, Zimbabwe.)

Intestinal anthrax is analogous to cutaneous anthrax but occurs on the intestinal mucosa. As in cutaneous anthrax, the organisms probably invade the mucosa through a preexisting lesion. Organisms spread from the mucosal lesion to the lymphatic system. In pulmonary anthrax, inhaled spores are transported by alveolar macrophages to the mediastinal lymph nodes, where they germinate and multiply to initiate systemic disease. Gastrointestinal and pulmonary anthrax are both more dangerous than the cutaneous form because they are usually identified too late for treatment to be effective.

Herbivorous animals, the primary hosts of *B. anthracis*, contract the infection by ingesting spores on forage plants; the spores are derived from soil or dust or are deposited on leaves by flies after feeding on an anthrax-infected carcass. If the spores enter a lesion in the gastrointestinal mucosa, they germinate and are taken into the bloodstream and lymphatics, finally producing systemic anthrax, which is usually fatal.

Symptoms prior to fulminant systemic anthrax may be absent or mild, consisting, for example, of malaise, low fever, and mild gastrointestinal symptoms in the case of gastrointestinal disease. During this phase the organism is multiplying and producing toxin in the regional lymph nodes and spleen. Released toxin causes breakdown of these organs probably of the spleen in particular. This causes the sudden onset of hyperacute illness with dyspnea, cyanosis, high fever, and disorientation, which progress in a few hours to shock, coma, and death. Although symptoms vary somewhat with the host species, this final acute phase is marked by a high-grade bacteremia. In humans, blood cultures are not always positive.

Bacillus Food Poisoning

Bacillus cereus can cause two distinct types of food poisoning. The diarrheal type is characterized by diarrhea and abdominal pain occurring 8 to 16 hours after consumption of the contaminated food. It is associated with a variety of foods, including meat and vegetable dishes, sauces, pastas, desserts, and dairy products. In emetic disease, on the other hand, nausea and vomiting begin 1 to 5 hours after the contaminated food is eaten. Boiled rice that is held for prolonged periods at ambient temperature and then quick-fried before serving is the usual offender, although dairy products or other foods are occasionally responsible. The symptoms of food poisoning caused by other *Bacillus* species (*B. subtilis*, *B. licheniformis*, and others) are less well defined. Diarrhea and/or nausea occurs 1 to 14 hours after consumption of the contaminated food. A wide variety of food types have proved responsible in recorded instances.

A *Bacillus* food poisoning episode usually occurs because spores survive cooking or pasteurization and then germinate and multiply when the food is inadequately refrigerated. The symptoms of *B. cereus* food poisoning are caused by a toxin or toxins produced in the food during this multiplication. Toxins have not yet been identified for other *Bacillus* species that cause food poisoning.

Structure and Classification

The family Bacillaceae, consisting of rod-shaped bacteria that form endospores, has two principal subdivisions: the anaerobic spore-forming bacteria of the genus Clostridium, and the aerobic or facultatively anaerobic spore-forming bacteria of the genus *Bacillus* frequently known as ASB (aerobic spore-bearers). Bacterial cells of *Bacillus* cultures are Gram positive when young, but in some species become Gram negative as they age.

Most *Bacillus* species are saprophytes. Table 15-1 lists the identifying characteristics of some of the species most likely to be encountered by the physician. Not only are *Bacillus* endospores resistant to hostile physical and chemical conditions, but also various species have unusual physiologic properties that enable them to survive or thrive in harsh environments, ranging from desert sands and hot springs to Arctic soils and from fresh waters to marine sediments. The genus includes thermophilic, psychrophilic, acidophilic, alkaliphilic, halotolerant, and halophilic representatives, which are capable of growing at temperatures, pH values, and salt concentrations at which few other organisms could survive.

TABLE 15-1 Basic Characteristics for Identification of Selected *Bacillus* Species*

Species ^b	Motility	Catalase Production	Parasporal Bodies	Lipid Globules in Protoplasm	Lecithinolin Reaction	Citrate Utilization	Anaerobic Growth	V-P Reaction	pH in V-P Medium < 6.0	Growth at 50° C	Growth at 60° C	Growth in 7% NaCl	Acid from AS Glucose	Acid + Gas from AS Glucose	Nitrate Reduction	Casein Hydrolysis	Starch Hydrolysis	Fringipane Utilization
Morphologic group 1																		
<i>B. megaterium</i>	v	+	-	+	-	+	-	-	v	-	-	+	+	-	v	+	+	v
<i>B. cereus</i>	+	+	-	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+
<i>B. cereus</i> subsp. <i>mycoides</i>	-	+	-	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+
<i>B. anthracis</i>	-	+	-	+	+	<	+	+	+	-	-	+	+	-	+	+	+	+
<i>B. thuringiensis</i>	+	+	-	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+
<i>B. licheniformis</i>	+	+	-	-	-	+	+	+	+	-	-	+	+	-	+	+	+	+
<i>B. subtilis</i>	+	+	-	-	-	+	-	+	+	<	<	+	+	-	+	+	+	+
<i>B. pumilus</i>	+	+	-	-	-	+	-	+	+	v	-	+	+	-	+	+	+	+
<i>B. firmus</i>	v	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+
<i>B. coagulans</i>	+	+	-	-	-	v	+	+	+	+	v	-	+	-	v	v	+	+
Morphologic group 2																		
<i>B. polymyxa</i>	+	+	-	-	-	-	-	+	+	v	-	-	+	+	+	+	+	+
<i>B. macerans</i>	+	+	-	-	-	v	+	-	+	+	-	-	+	+	-	+	+	+
<i>B. circulans</i>	v	+	-	-	-	v	v	-	+	v	-	v	+	-	v	v	+	+
<i>B. stearothermophilus</i>	+	v	-	-	-	-	-	-	+	+	-	-	+	-	v	v	v	+
<i>B. alvei</i>	+	+	-	-	-	-	+	+	+	-	-	-	+	-	+	+	+	n
<i>B. laterosporus</i> ^c	+	+	-	-	(+)	-	+	-	+	-	-	-	+	-	+	+	+	n
<i>B. brevis</i>	+	+	-	-	v	-	-	-	v	v	-	+	-	v	+	-	n	n
Morphologic group 3																		
<i>B. sphaericus</i>	+	+	-	-	-	v	-	-	-	-	v	-	-	v	-	v	-	n

* V-P, Voges-Proskauer; AS, ammonium salt; +, more than 85 percent of strains examined by Gordon et al. (See References) were positive; -, more than 85 percent of strains negative; v, variable; n, test not applicable; (+), under colony which must be scraped off to see positive reaction.

^b Species grouped according to the classification scheme of Gordon et al. Morphologic group 1: sporangium not swollen by spore; spore ellipsoidal or cylindrical, central or terminal; Gram positive. Morphologic group 2: sporangium swollen by ellipsoidal spore; spore central or terminal; Gram variable. Morphologic group 3: sporangium swollen by spore; spore spherical, subterminal, or terminal; Gram variable.

^c Sporangium and spore have characteristic canoe shape.

Figure 15-2 shows the structure of a generalized *Bacillus* endospore (details of the structure differ from species to species). One spore is produced per vegetative cell. The central protoplast, or germ cell, carries the constituents of the future vegetative cell, accompanied by dipicolinic acid, which is essential to the heat resistance of the spore. Surrounding the protoplast is a cortex consisting largely of peptidoglycan (murein), which is also important in the heat and radiation resistance of the spore. The inner layer, the cortical membrane or protoplast wall, becomes the cell wall of the new vegetative cell when the spore germinates. The spore coats, which constitute up to 50 percent of the volume of the spore, protect it from chemicals, enzymes, etc.

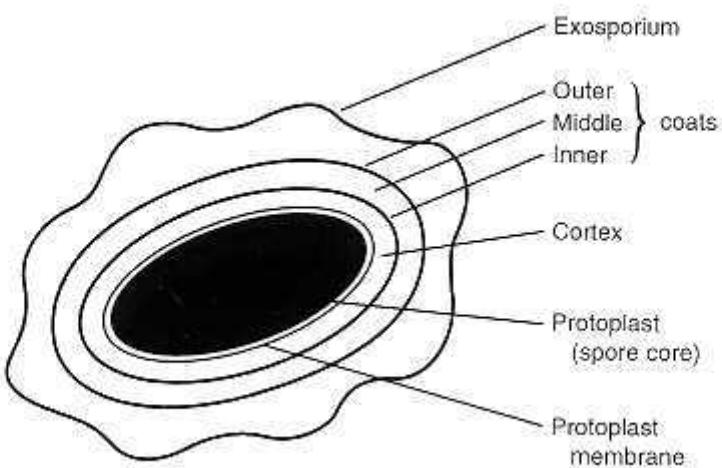


FIGURE 15-2 Cross section of a *Bacillus* spore.

The events involved in sporulation of vegetative cells and in germination of spores are complex and are influenced by factors such as temperature, pH, and the availability of certain divalent cations and carbon- and nitrogen-containing compounds. Spores formed under different conditions have different stabilities and degrees of resistance to heat, radiation, chemicals, desiccation, and other hostile conditions.

Pathogenesis

The pathogenicity of *B. anthracis* depends on two virulence factors: a poly- γ -D-glutamic acid polypeptide capsule, which protects it from phagocytosis by the defensive phagocytes of the host, and a toxin produced in the log phase of growth. This toxin consists of three proteins: protective antigen (PA) (82.7 kDa), lethal factor (LF) (90.2 kDa), and edema factor (EF) (88.9 kDa). Host proteases in the blood and on the eukaryotic cell surface activate protective antigen by cutting off a 20-kDa segment, exposing a binding site for LF and EF. The activated 63 kDa PA polypeptide binds to specific receptors on the host cell surface, thereby creating a secondary binding site for which LF and EF compete. The complex (PA+LF or PA+EF) is internalized by endocytosis and, following acidification of the endosome, the LF or EF cross the membrane into the cytosol via PA-mediated ion-conductive channels. This is analogous to the A-B structure-function model of cholera toxin with PA behaving as the B (binding) moiety (Fig. 15-3). EF, responsible for the characteristic edema of anthrax, is a calmodulin-dependent adenylate cyclase. (Calmodulin is the major intracellular calcium

receptor in eukaryotic cells.) The only other known bacterial adenylate cyclase is produced by *Bordetella pertussis* (see Ch 31), but the two toxins share only minor homologies. LF appears to be a zinc-dependent metalloprotease though its substrate and mode of action have yet to be elucidated.

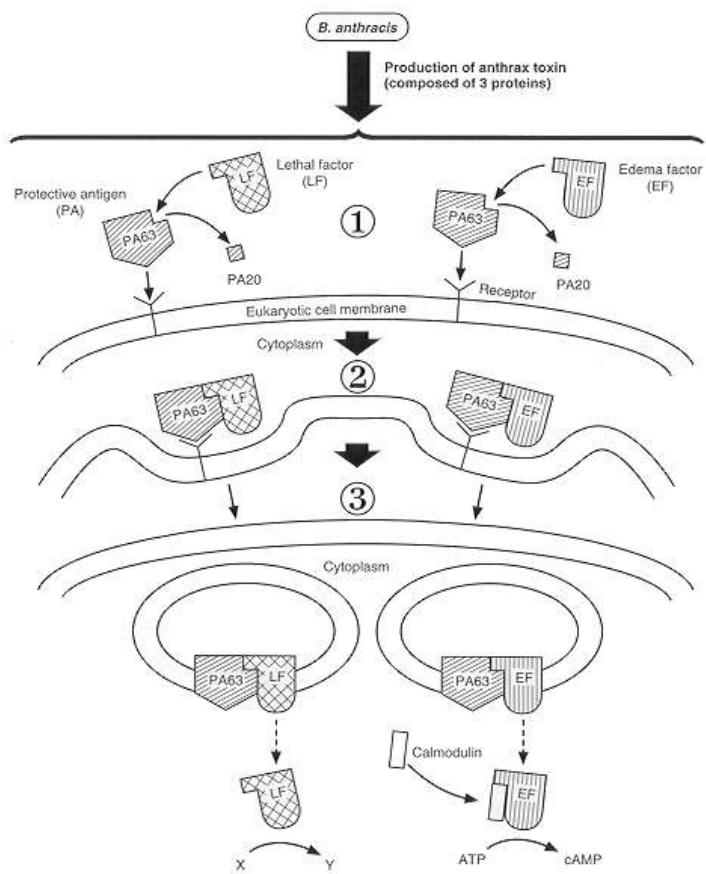


FIGURE 15-3 Mechanism of action of the anthrax toxin. The toxin is composed of three proteins. Protective antigen (PA) binds to an appropriate site on the host cell membrane. A cell surface protease cleaves off a 20-kDa piece from the protective antigen and thereby exposes a secondary binding site for which lethal factor (LF) and edema factor (EF) compete. The complex (PA+LF or PA+EF) is internalized by receptor-mediated endocytosis, and acidification of the endosome results in the transfer of the LF or EF across the endosome membrane into the cytosol where they carry out their catalytic actions. (Model by S.H. Leppla, Ph.D., Laboratory of Microbial Ecology, National Institutes of Health, Bethesda, MD.)

The toxin and capsule of *B. anthracis* are encoded on two large plasmids called pXO 1 (110 MDa) and pXO2 (60 MDa), respectively. Strains lacking either of these plasmids have greatly reduced virulence (Fig. 15-4). The attenuated live vaccine strain developed by Sterne in 1937, which is still the basis of most anthrax vaccines for livestock, lacks pXO2 and is therefore Cap- Tox+. The protection afforded by such vaccines apparently is related primarily to antibodies specific for the protective antigen component of the toxin. In contrast, the attenuated vaccine strains developed by Pasteur 110 years ago were inadvertently cured of pXO1 (by subculturing at 42° to 43°C); these Pasteur strains are therefore Cap+ Tox-. Strains of this type do not induce protective immunity; the partial effectiveness of Pasteur's vaccines is now believed to have been due to the

residual uncured (Cap^+ Tox^+) cells they contained, and this would also explain the partial virulence of these strains.

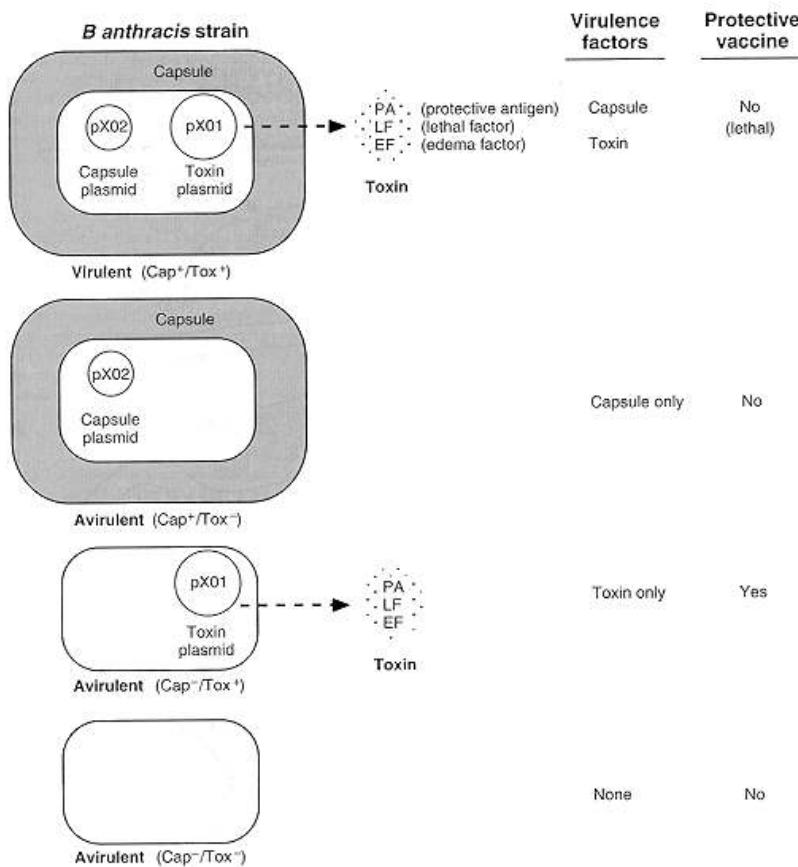
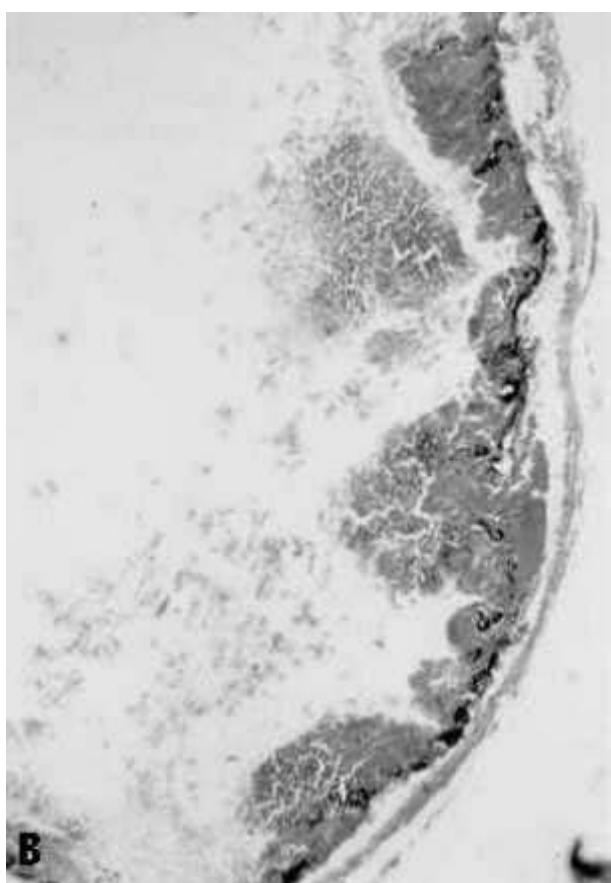


FIGURE 15-4 Genetics of virulence factor production by *B. anthracis*. Plasmids pXO1 and pXO2 encode, respectively, the anthrax toxin and capsule. Curing the bacteria of pXO1 produces an encapsulated, nontoxicogenic strain that is nonprotective. Curing of pXO2 produces a toxigenic nonencapsulating strain that can be used as a protective vaccine. Production of protective antigen is essential for a strain to be protective.

The only other *Bacillus* species for which virulence factors have been identified is *B. cereus*. A 38 to 46-kDa protein complex has been shown in animal models to cause necrosis of the skin or intestinal mucosa (Fig. 15-5), to induce fluid accumulation in the intestine, and to be a lethal toxin. This protein is believed to be responsible for the necrotic and toxemic nature of severe *B. cereus* infections and for the diarrheal form of food poisoning. *Bacillus cereus* also produces two hemolysins; one of these, cereolysin (58 kDa), is a potent necrotic and lethal toxin. Although this toxin is neutralized by serum cholesterol, it probably contributes to the pathogenesis of *B. cereus* infections. Little is known about the other hemolysin at present. Phospholipases produced by *B. cereus* may act as exacerbating factors by degrading host cell membranes following exposure of their phospholipid substrates in wounds or other infections. The agent responsible for the emetic type of *B. cereus* food poisoning has not been clearly identified. The emesis may be induced by breakdown products resulting from the action of one or more *B. cereus* enzymes on the food.



A



B

FIGURE 15-5 Necrosis of rabbit ileal mucosa 4 hours after introduction of a toxigenic cell-free culture filtrate of *B cereus*. (A) Gross appearance of the luminal

surface of the ileum compared with a section of control ileum. (B) Histologic appearance of a cross-section of the toxin-exposed ileum. (From Turnbull PCB: Studies on the production of enterotoxins by *Bacillus cereus*. *J Clin Pathol* 29:941, 1976, with permission.)

Host Defenses

Anthrax has been documented in a wide variety of warm-blooded animals. Some species, such as rats, chickens, and dogs, are quite resistant to the disease, whereas others (notably herbivores such as cattle, sheep, and horses) are very susceptible. Humans have intermediate susceptibility. The specific mechanisms of resistance in the more resistant species are not known.

Protective immunity against anthrax requires antibodies against components of anthrax toxin, primarily protective antigen. Both the noncellular human vaccines and live-spore animal vaccines confer protection by eliciting antibodies to protective antigen. The poly-g-D-glutamic acid capsule of *B anthracis* is poorly immunogenic, and antibodies to the polysaccharide and other components of the cell wall are not protective.

Nothing is known about immune responses to food poisoning or other types of infections with *Bacillus* species other than *B anthracis*. These types of infection are rare, and effective vaccines against them have not been developed.

Epidemiology

The ultimate reservoir of *B anthracis* is contaminated soil, in which spores remain viable for long periods. Herbivores, the primary hosts, become infected when foraging in a contaminated region. Because the organism does not depend on an animal reservoir, it cannot readily be eradicated from a region, and anthrax remains endemic in many countries. Humans become infected almost exclusively through contact with infected animals or animal products. Human anthrax is traditionally classified as either nonindustrial or industrial anthrax, depending on whether the disease is acquired directly from animals or indirectly during handling of contaminated animal products. Nonindustrial anthrax usually affects people who work with animals or animal carcasses, such as farmers, veterinarians, knackers, and butchers, and is almost always cutaneous. Industrial anthrax, acquired from handling contaminated hair, hides, wool, bone meal, or other animal products, has a higher chance of being pulmonary as a result of the inhalation of spore-laden dust.

The development of an effective animal vaccine in the 1930s, together with improved factory hygiene, introduction of procedures for sterilizing imported animal products, replacement of animal products with man-made alternatives, and the availability since the mid-1960s of a human vaccine, has resulted in a greatly reduced incidence of the disease in North America. Human anthrax is now very rare in the United States. However, major epidemics still break out in endemic countries, normally following an outbreak in livestock. Nonendemic countries must remain alert for episodes of anthrax arising from imported animal products.

Diagnosis

The clinical diagnosis of anthrax is confirmed by directly visualizing or culturing the anthrax bacilli. Fresh smears of vesicular fluid, fluid from under the eschar, blood, lymph node or spleen aspirates, or (in meningitic cases) cerebrospinal fluid are stained with polychrome methylene blue (M'Fadyean's stain) and examined for the characteristic square-ended, blue-black bacilli surrounded by a pink capsule (Fig. 15-6). (It should be remembered that *B. anthracis* organisms are not invariably detected in stained blood smears of humans dying of anthrax.) Alternatively, the bacilli may be cultured from these specimens and checked for sensitivity to the anthrax gamma phage, for penicillin sensitivity, and for capsule formation. Colonies grown overnight at 37°C on blood agar are gray or white, nonhemolytic, with a dry, ground-glass appearance; they are at least 3 mm in diameter and sometimes have tails (Fig. 15-7). Capsules can be seen in polychrome methylene blue-stained smears of cultures grown on nutrient agar containing 0.7 percent sodium bicarbonate and incubated overnight under CO₂ (e.g., in a candle jar); encapsulated colonies are mucoid. Alternatively, 2 ml of blood (such as commercial defibrinated horse blood) inoculated with a pinhead quantity of material from a suspected colony and incubated at 37°C yields readily demonstrable encapsulated bacilli in 6 hours. Culturing may be unsuccessful if the patient has been treated with antibiotics.

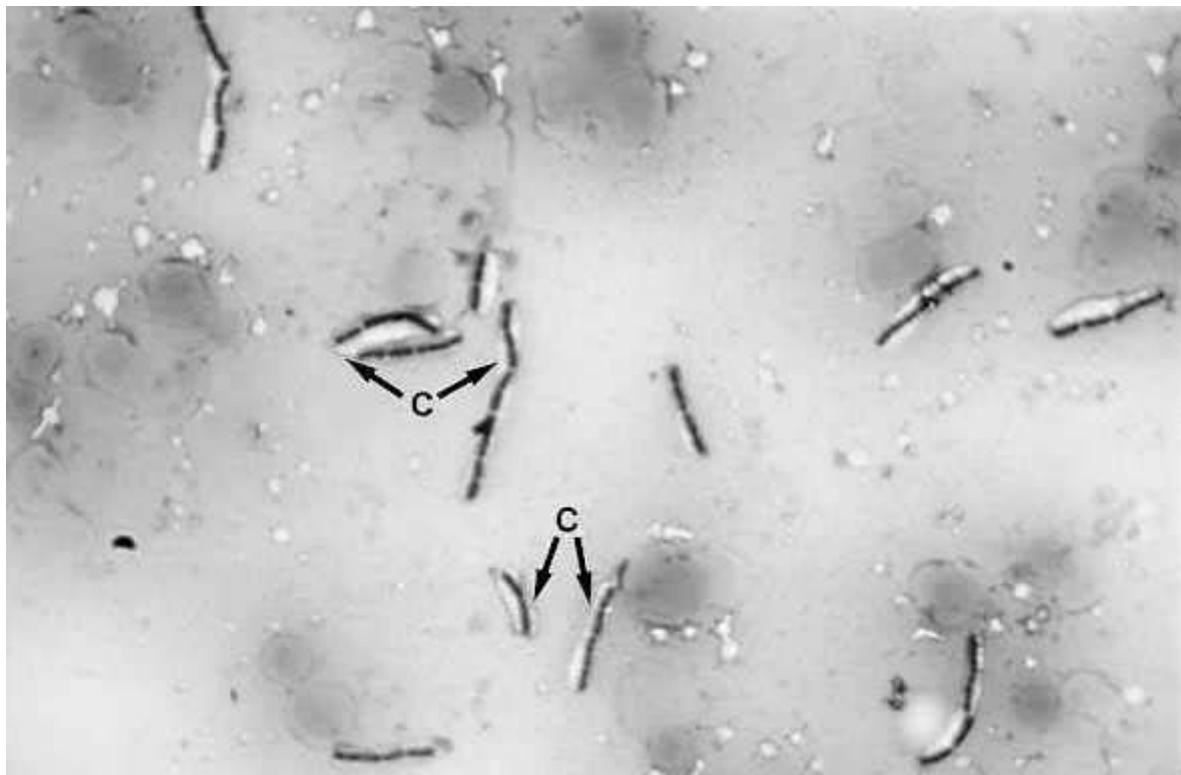


FIGURE 15-6 Blood smears from a guinea pig that died of anthrax, stained with M'Fadyean stain (polychrome methylene blue). The capsule (C) is pink around the dark-blue bacilli. Although not obvious from this photograph, anthrax bacilli frequently have square ends.

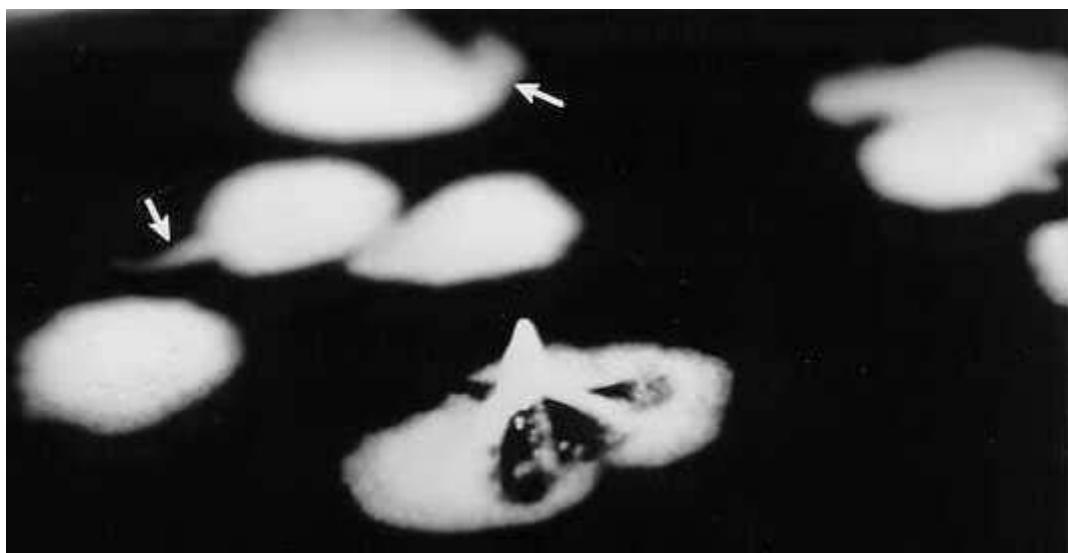


FIGURE 15-7 Colonies of *B. anthracis* on a blood agar plate. Note the characteristic tackiness of colonies that allow them to be teased upright with a loop (foreground) and the characteristic tailing seen in the background (arrows). (Photograph kindly supplied by R.W. Charlton. From Turnbull PCB, Kramer JM, Melling J: *Bacillus*. p. 187. In Parker MT, Duerden BI (eds): *Systematic Bacteriology*. Topley and Wilson's *Principles of Bacteriology, Virology and Immunity*. Vol. 2. Edward Arnold, Sevenoaks, England, 1990, with permission.)

Isolation of *B. anthracis* from old specimens or from animal or environmental material being examined for public health purposes is more difficult, particularly if, as is often the case, *B. cereus* or other *Bacillus* species are present in substantial numbers. The specimen should be examined both unheated and heated to 60°C to 65°C for 15 min with subculture to both blood or nutrient agar and specialized selective agars. Very rarely it may be necessary to use mouse or guinea pig inoculation to isolate *B. anthracis*. Up to about 0.2 ml of the specimen (or an aqueous extract of the specimen) is injected subcutaneously into a mouse, or intramuscularly or subcutaneously in a guinea pig (more sensitive than a mouse); the encapsulated bacilli can be seen in a smear of blood aspirated from the heart of the animal at death, and the bacteria are readily observed in and isolated from this blood. If soil samples are being used, the animals should be injected 24 hours earlier with tetanus and gas gangrene antitoxin.

When a specimen from an individual not suspected clinically of having anthrax yields substantial numbers of Gram-positive bacilli, the specimen should be cultured and tested as shown in Figure 15-8 to determine the *Bacillus* species present. The most common *Bacillus* species may be identified by the characteristics in Table 15-1. Incrimination of a *Bacillus* species as the cause of an infection is usually based on its presence in large numbers at the infection site, especially in the absence of other known pathogens. Since *Bacillus* species are common environmental organisms, their presence in small numbers is not generally considered significant. For this reason, the use of selective or enrichment systems for isolating clinically relevant, nonanthrax *Bacillus* species is confined to just a few situations, such as the retrospective examination of feces several days after a food poisoning incident (by which time the offending *Bacillus* organism may be present in only small numbers).

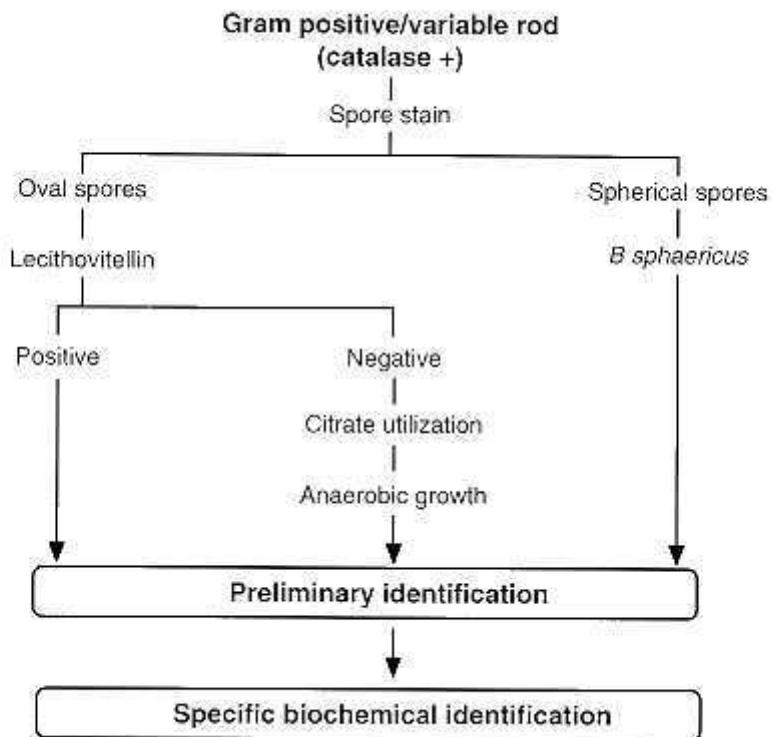


FIGURE 15-8 Flow chart for identification of principal *Bacillus* species.

Control

To comprehend the strategies used to control anthrax, it is important to understand the cycle of infection in susceptible animals. As a susceptible animal with anthrax approaches death, its blood contains as many as 10^9 bacilli/ml (depending on the species). Necrosis of the walls of small blood vessels during the acute phase of the illness leads to hemorrhages and to characteristic bloody exudations from the mouth, nose, and anus—a highly diagnostic sign. These exudates carry vast numbers of the bacilli, which sporulate on exposure to air and produce a heavily contaminated environmental site that is potentially capable of infecting other animals for many years.

Because sporulation of *B. anthracis* requires oxygen and therefore does not occur inside a closed carcass, regulations in most countries forbid postmortem examination of animals when anthrax is suspected. The vegetative cells in the carcass are killed in a few days by the process of putrefaction. Nevertheless, in the case of livestock, legislation invariably requires that the carcass be burned or buried in quicklime (calcium oxide). However, it is becoming increasingly apparent in the new era of sensitivity about environmental contamination that implementation in the past (sometimes many decades) of the order to bury in quicklime has left us a legacy of burial sites which are contaminated with viable anthrax spores and it is to be hoped that this instruction will be removed from veterinary public health orders.

Livestock in endemic areas are effectively protected by yearly inoculations with a vaccine made from spores of a live attenuated strain (see above). Noncellular vaccines

for human use are available for individuals in high-risk occupations. They appear to have contributed to the decline in incidence of industrial anthrax since they became available in the 1960s, but animal studies suggest that there are limitations to their ability to protect against anthrax. The human vaccine available in the United States is an aluminum hydroxide-adsorbed cell-free filtrate of a *B anthracis* culture grown to maximize the yield of protective antigen and minimize the quantities of lethal factor, edema factor, and other unwanted metabolites.

Bacillus anthracis is susceptible to penicillin and to almost all other broad-spectrum antibiotics. Because it is easily recognized, cutaneous anthrax is almost always treated early and cured. Gastrointestinal and pulmonary anthrax infections are difficult to identify before the fulminant phase and therefore carry a high mortality. In uncomplicated anthrax cases, adequate treatment consists of 500 mg of penicillin V taken orally every 6 hours for 5 days, or 600 mg (1 million units) of procaine penicillin administered intramuscularly every 12 to 24 hours for 5 days. In severe cases, 1,200 mg (2 million units) of penicillin G should be administered intravenously every 6 hours, reverting to the intramuscular regime of 600 mg every 12 to 24 hours once recovery starts. If pulmonary anthrax is suspected, continuous-drip administration is advisable. Tetracyclines (tests in animals indicate doxycycline is good), chloramphenicol, gentamicin, or erythromycin may be used if the patient has penicillin hypersensitivity. The fluoroquinolone, ciprofloxacin, has also been shown to be effective in monkeys and guinea pigs and would be expected to be effective in treatment of cases of human anthrax.

Avoidance of other types of *Bacillus* infections is largely a matter of observing proper hygiene. *Bacillus cereus* and its close relatives *B thuringiensis* and *B mycoides* produce potent β -lactamases and thus are not responsive to penicillin, ampicillin, or the cephalosporins. They are mostly resistant to trimethoprim as well. These species are generally sensitive to standard empirical treatment with an aminoglycoside combined with vancomycin and to chloramphenicol, erythromycin, tetracycline, clindamycin, and sulfonamides.

Bacillus food poisoning, like all types of food poisoning, can largely be prevented by proper food handling. Food should be cooked adequately; cooked food should not be recontaminated from uncooked food (separate utensils and cutting surfaces should be used for cooked and uncooked food); and, of particular importance, cooked food should be stored under proper refrigeration.

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