

Brucella, Francisella and Yersinia pestis **Gram negative rods of Zoonoses**

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PAMB 650/720 Medical Microbiology Lectures: 50

Lectures: 50-51

TEACHING OBJECTIVES:

1. Know the general morphology and physiology of the organisms
2. Know epidemiology and clinical symptoms
3. Understand the mechanisms pathogenesis

READING:

Murray, *et al.*: Medical Microbiology, 6th ed. Chapter 36, *Francisella* and *Brucella*, pages 357-363. Chapter 30, *Yersinia pestis*, pages 311-313.

Zoonosis refers to a disease primarily of animals which can be transmitted to humans as a result of direct or indirect contact with infected animal populations.

BRUCELLOSIS

Brucellosis is primarily a disease of animals with a predilection for organs rich in erythritol (breast, uterus, placenta, epididymis). The organism localizes in these animal organs and causes infertility, sterility, mastitis, abortion, or carrier state in non-human animals.

Brucellosis is common where significant disease is found among the domestic animal population. The Centers for Disease Control listed as high risk for potential risk of exposure to *Brucella* the following areas; Mediterranean Basin (Portugal, Spain, Southern France, Italy, Greece, Turkey, and North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East. Unpasteurized cheeses, from these areas are a particular risk for tourists. Brucellosis is often referred to as undulant fever, Malta fever, and Mediterranean remittent fever.

The bacteria can penetrate intact skin. Therefore, two types of patient populations are seen. Humans in close contact with infected animals (slaughterhouse workers, veterinarians, farmers, dairy workers) are at risk of developing brucellosis and individuals who ingest unpasteurized dairy products contaminated with *Brucella*.

Four different species of *Brucella* infect humans and each bacterial species is animal host specific: *B. abortus* (cattle), *B. suis* (swine), *B. melitensis* (goats/sheep) and *B. canis* (dogs).

Morphology and physiology:

Brucella are poorly staining Gram-negative, small coccobacilli that grows slowly (fastidious) on 5% sheep blood agar, or chocolate agar. Isolates typically do not grow on MacConkey agar or Eosin methylene blue (EMB) agar. *Brucella* appears mostly as single cells and look like “fine sand”. These organisms are **nonhemolytic** and **nonmotile**. *Brucella* species are strict aerobes, except for some strains of *B. abortus* which require 5 % CO₂ on primary isolation. They are catalase, oxidase and urease positive. The urease test is specifically useful for characterization of the *Brucella*. *B. suis* and some *B. melitensis* strains produce a rapid

reaction that can be observed within 5 min of inoculation on a Christensen's urea slant. The other *Brucella* species produce a positive reaction after overnight incubation.

Public Health Aspects:

Animal Sources:

Four species of *Brucella* cause brucellosis when transmitted to humans.

1. *B. abortus* primarily from cattle
2. *B. melitensis* from sheep, goats and camels
3. *B. suis* from pigs
4. *B. canis* from dogs

Patient Populations:

2 distinct populations

1. Individuals who work with or are exposed to unvaccinated animals. This group is exposed via direct skin contact and inhalation. Farmers, veterinarians, slaughterhouse workers, hunters and laboratory workers contract brucellosis in this way.
 - a. *B. abortus*
 - b. *B. suis*
2. Individuals who ingest unpasteurized dairy product are exposed in this way. Tourists and individuals from areas with unvaccinated animals develop brucellosis via this route.
 - a. *B. melitensis*

Epidemiology:

There are 100-200 cases of brucellosis seen in the US, although the worldwide incidence is significantly larger. *B. suis* is endemic in the feral swine population in 35 states including South Carolina.

Symptoms:

In the animal host brucellosis may be asymptomatic or cause only a mild disease. Brucellosis in animals may also lead to sterility, abortions and a carrier state in the non-human animal.

In humans symptoms can appear up to 2 months after exposure. During the **acute phase** (<8 weeks from illness onset) the patient presents with nonspecific and "flu-like" symptoms. These include fever, sweats, malaise, anorexia, headache, myalgia, back pain, chills, fatigue, weight loss, arthralgias, and non-productive cough. The fever may be intermittent fever (undulant fever). As the disease advances into the **undulant form or advanced disease** (<1 year from illness onset) symptoms include undulant fevers, arthritis, and epididymo-orchitis in males. **Chronic disease** (>1 year from onset) can mimic miliary tuberculosis with suppurative lesions in the liver, spleen, and bone. The patient may have recurrent fevers, arthritis, depression, and chronic fatigue syndrome. Brucellosis may lead to granulomatous hepatitis, peripheral arthritis, leucopenia, thrombocytopenia, meningitis, and endocarditis.

The clinical spectrum of disease is dependent on the infecting organism. The following table is a summary.

Organism	Animal reservoir	Human disease	Complications
<i>B. abortus</i>	Cattle	mild suppurative febrile infection	Rare
<i>B. canis</i>	Dogs	mild suppurative febrile infection	Rare
<i>B. suis</i>	Swine	Prolonged disease which may lead to the formation of destructive lesions of the lymphoreticular organs and kidney	
<i>B. melitensis</i>	goats/sheep	Severe and recurring disease	high incidence of serious complications

Pathogenesis:

In the host, *Brucella* survives as **facultative intracellular pathogens within the reticuloendothelial system.** Following penetration of the mucosal epithelium (ingestion/inhalation routes, abraded skin, or the conjunctiva) the bacteria are transported via macrophages to the regional lymph, leading to acute lymphadenitis. *Brucella* inhibits the myeloperoxidase-H₂O₂-halide antibacterial systems in neutrophils hampering degranulation. In the macrophage *Brucella* inhibits phagosome-lysosome fusion allowing the bacteria to survive and multiply. The systemic spread and multiplication of *Brucella* in lymph nodes, spleen, liver, bone marrow, mammary glands, and sex organs occurs via macrophages. Once distributed to other organs the bacteria induce the formation of granulomas and/or micro abscesses. The ability of *Brucella* to survival in macrophages is responsible for the establishment of chronic infections. *Brucella* species do not produce exotoxins.

Diagnosis:

Diagnosis is based on presence of clinical presentation (undulant fever, myalgia, arthralgias) and the history of exposure (contact with animals or consumption of unprocessed material from infected animals). Definitive diagnosis can be made by culture (culture positive blood, bone, tissue or abscess fluid). The (fastidious) organisms grow very slowly. Serological testing such a serum agglutination test (SAT) can be a valuable diagnostic tool. A four-fold rise in the SAT titer between the acute and convalescent phase sample is indicative of brucellosis.

Prevention and treatment:

Prolonged treatment with tetracycline, doxycycline, or trimethoprim-sulfamethoxazole in combination and rifampin or gentamicin for 6 weeks is used to prevent reoccurring infections. Control measures include animal vaccination and avoidance of infected material (*e.g.*, unpasteurized dairy products).

TULAREMIA

Francisella tularensis is the causative agent of tularemia. Its primary reservoirs are **rabbits, deer, and rodents;** however *F. tularensis* has been isolated from over 100 species of wild animals. Humans commonly acquires tularemia via the **insect bites** (ticks primarily, but

also deer flies, mites, black flies, or mosquitoes) or by **handling infected animal tissues, inhalation of aerosols or ingestion of contaminated food and water.**

F. tularensis is a gram negative coccobacillus and **two major biotypes** of *F. tularensis* are seen. **Biotype A (*F. tularensis* subsp. *tularensis*) is found only in North America** and is more virulent, **Biotype B (*F. tularensis* subsp. *holarctica*)** is found in Europe and Asia and is less virulent. Human disease (rabbit or deer fly fever) is characterized by a focal ulcer at the site of entry of the organisms and enlargement of the regional lymph nodes. It takes as few as 10-50 organisms to infect via the aerosol or intradermal route.

Morphology and physiology:

F. tularensis is a small, Gram-negative, nonmotile, encapsulated, pleomorphic coccobacillus (short rod). The organism grows poorly on most lab media and requires media containing glucose and **cysteine**, for isolation. *F. tularensis* will grow on chocolate agar, and buffered charcoal yeast extract agar. Organisms do not grow on MacConkey or eosin-methylene blue (EMB) agars.

The organism is aerobic and slow growing so prolonged incubation times are needed (48 hours minimum, hold for 5 days) at 35-37⁰ C. Colony morphology at 24 hours is too small to be seen. At 48 hours, colonies are 1 to 2 mm in diameter, white to gray to bluish-gray, opaque, flat, and smooth with an entire edge and shiny surface.

Biochemical screening tests:

- Oxidase-negative
- Weakly catalase-positive (although may be negative)
- Urea-negative
- Nitrate-negative
- Non-motile
- Beta-lactamase-positive

Public Health-Modes of Infection:

In the United States, biting arthropods such as ticks and biting flies are the most important vectors.

Outbreaks in the United States have been associated with infected rabbits, muskrats, prairie dogs sold as pets, and the use of lawnmowers and brush cutters. Tularemia has been reported in individuals who sustained bites from hamsters and cats. Occupations at increased risk for transmission of tularemia include laboratory workers, landscapers, farmers, veterinarians, hunters, trappers, cooks, and meat handlers. Naturally occurring cases are seen during the summer months when people are exposed to ticks and biting flies and during hunting season.

Epidemiology and symptoms:

Tularemia occurs routinely in all 50 of the United States (~100 cases per year) primarily in the south central (Arkansas, Missouri and Oklahoma) and western states. As few as 10-50 bacilli will cause disease in humans if inhaled or introduced intradermally, whereas a very large inoculum (~10⁸ organisms) is required for the oral route of infection.

The incubation period is 3-10 days with a range of 1-14 days. The clinical manifestations of tularemia can be divided into 5 groups: **Ulceroglandular form** is most common (45-85%) in which a painful ulcerating papule which has a necrotic center and raised periphery develops at the site of infection, **glandular** (without ulcer), **typhoidal**, **pneumonic**, **oculoglandular**, and **oropharyngeal/ gastrointestinal**. Ingestion of infected meat or water can result in oropharyngeal or gastrointestinal tularemia

The main difference between these groupings is the involvement of the skin/mucous membranes and associated lymphadenopathy. In ulceroglandular tularemia a local painful cutaneous lesion forms at site of inoculation and the papule ulcerates within a few days. Ulceration is accompanied with fever, tender regional lymphadenopathy, and flu-like symptoms (chills, myalgias, malaise, arthralgias, headache, and anorexia).

Typhoidal tularemia (sepsis) is distinguished by clinical findings of a high fever, splenomegaly, and hepatomegaly. Patients who have typhoidal form of tularemia also commonly have associated gastrointestinal and pulmonary symptoms as well as the typical flu-like symptoms. Typhoidal tularemia can result from pulmonary or gastrointestinal tularemia.

Pneumonia is a complication in 30% of patients with ulceroglandular tularemia and 80% of patients with typhoidal tularemia. Pneumonic tularemia results from exposure to aerosolized particles of *F. tularensis* or from hematogenous spread. Patients often present with community-acquired atypical pneumonia nonresponsive to conventional antibiotic therapy

Pathogenesis:

F. tularensis is a **facultative intracellular parasite that multiplies predominately in macrophages**. The **capsule** of the organism renders it resistant to killing by complement. Uptake by macrophages involves serum complement and receptors for C3. Once inside the macrophage *F. tularensis* arrests maturation of the phagosome (LPS-O antigen is important for intracellular survival) and enters into the cytosol where it multiplies to high levels and triggers apoptosis of the macrophage which results in the release of the bacteria into surrounding tissue. Little is known about specific pathogenesis factors.

Diagnosis:

F. tularensis is difficult to visualize in direct smears by Gram stain, but direct fluorescent-antibody (DFA) staining will improve visualization. Lymph node aspirates and sputum (pneumonic) are cultured on chocolate agar or **buffered charcoal yeast extract agar (BCYE)**. Blood cultures are often negative (7days incubation or longer). The organism grows very slowly and hence must be **incubated for several days**. Serum antibody testing is the most common for identification of *F. tularensis*. Culture of *F. tularensis* is not generally done in the clinical laboratory and requires utilization of a biosafety cabinet for processing of samples.

Prevention and treatment:

Streptomycin is the drug of choice, **gentamycin** is an alternative. A live attenuated vaccine is available but it is not completely effective. One must avoid handling infected animals, watch out for ticks and utilize clean water supplies.

Yersinia

Three strain of *Yersinia* can cause human disease, *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis*. *Y. enterocolitica* and *Y. pseudotuberculosis* cause gastroenteritis, *Y. pestis* causes plague.

Morphology and physiology:

Yersinia pestis is a pleomorphic, Gram-negative, bipolar staining, facultatively anaerobic, bacillus. Optimal temperature for growth is 28°C. It is a facultative intracellular parasite. This organism grows well on standard laboratory media (sheep blood agar). It is non-hemolytic, non-motile, oxidase and urease negative.

Epidemiology, transmission and symptoms:

The three documented pandemics of plague (Black Death) have been responsible for the death of hundreds of millions of people. Today, sporadic infections still occur. In the U.S., animal (**sylvatic**) plague occurs in a number of western states, usually in small rodents, and carnivores that feed on these rodents. Human cases (**Urban plague**) occur occasionally, particularly in the southwestern US. This is generally spread through rats in the urban environment.

Humans are infected by carrier rodent fleas or by contact with infected animals. The flea acquires the *Y. pestis* organisms during a blood meal from infected rodents. These organisms lose their capsule (in the flea), multiply in the intestinal tract. While feeding on a human host the flea regurgitates some of the organisms into the wound. After the incubation period of 2-7 days, symptoms will appear.

During the incubation period the bulk of non-capsular organisms are phagocytosed and destroyed by neutrophils. However, few organisms are taken up by monocytes that are unable to kill them and the organisms resynthesize their capsule and multiply. The encapsulated organisms, when released from monocytes are resistant to phagocytosis and killing by neutrophils. The resulting infection spreads to the draining lymph nodes which become hot, swollen, tender and hemorrhagic, giving rise to the characteristic black buboes (**bubonic plague**). Inguinal, axillary, or cervical lymph nodes are the most commonly noted as grossly swollen and sore. Septicemia (septicemic plague) will occur in 80% of the patients.

The organism spreads into the spleen, liver and lungs resulting in pneumonia. While in circulation, the organism causes diffuse coagulation resulting in intra-vascular thrombi and purpuric lesions all over the body. If untreated, the infection has a very high (up to 90%) mortality. The organisms are exhaled in cough droplets, infect other humans in close proximity and cause **pneumonic** plague, which is more difficult to control and has 100% mortality.

Pathogenesis: Many pathogenic factors, including exotoxins, play direct and indirect roles in *Y. pestis* pathogenesis.

Yops: A group of 11 proteins, which are coded by plasmids, are essential for pathogenesis and are responsible for cytotoxicity, inhibition of platelet aggregation, phagocyte migration and engulfment. These proteins target dendritic cells, macrophages and neutrophils, but do not affect T and B lymphocytes.

Envelope (F-1) antigen: It is a protein-polysaccharide capsule that is highly expressed at 37°C in the mammalian host but not in the flea and is anti-phagocytic.

Plasminogen activator (fibrinolysin): Plasminogen activator (fibrinolysin) promotes the dissemination of the organism.

Diagnosis:

Diagnosis is based on appearance of buboes. The diagnosis is confirmed by culture of a lymph node aspirate. Extreme caution is warranted in handling of the specimen, as it is highly infectious. *Y. pestis* grows well on most standard laboratory media. Fluorescent-antibody testing is available.

Prevention and Treatment:

Hospitalization and strict isolation are the rule. Streptomycin and tetracycline are highly effective. An effective formalin-killed vaccine is available but is recommended only for people at a high risk. The disease is internationally quarantined and reporting of cases is mandatory. Control of urban plague is based upon flea and rodent control.

Listeria, Erysipelothrix and Bacillus **Gram positive rods of Zoonoses**

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READING:

Murray, *et al.*: Medical Microbiology, 6th ed. Chapter 25, *Listeria* and *Erysipelothrix*, pages 255-260. Chapter 24. *Bacillus*, pages 247-253.

LISTERIOSIS

Listeriosis is a nationally notifiable disease. *Listeria* contains 6 species only 1 of which is a human pathogen, *L. monocytogenes* (human pathogen). Listeriosis is caused by the ingestion of contaminated foods resulting in an **acute febrile gastroenteritis**. In pregnant women listeriosis can result in spontaneous abortions and a mild flu-like illness. Neonatal disease is contracted transplacentally or during delivery. Listeriosis can also cause meningitis and sepsis in the elderly, and immunocompromised.

Morphology and Physiology:

Listeria monocytogenes is a **facultative intracellular**, Gram-positive coccobacillus which often grows in short chains. The organism is **motile at 25⁰ C** with a unique **end-to-end tumbling** which is not seen at 37⁰ C. The bacteria grow at 4⁰ C and this trait is used to enrich specimens. *L. monocytogenes* can also grow in high salt concentrations. The organism forms beta hemolytic colonies on blood agar plates. Cold enrichment (storage of the sample for 24-48 hours at 4⁰ C) is a common means select for *Listeria*.

Epidemiology and symptoms:

L. monocytogenes is a ubiquitous organism found in the soil, vegetation, water, and in the gastrointestinal tract of animals. *L. monocytogenes* has many opportunities to enter the food-production and food-processing environments. Recovery rates for the organism are common from raw vegetables, unpasteurized milk, fresh soft cheese, and meats (including fresh-frozen and processed chicken and beef in supermarkets and delicatessens). The major mode of transmission is through ingestion of contaminated food and inadequately pasteurized milk (or milk contaminated post-pasteurization). These foods include ready-to-eat delicatessen meats, hot dogs, pate, soft cheeses, and other dairy products.

Exposure to the organism can lead to full range of diseases from asymptomatic disease, gastroenteritis, and meningitis in healthy adults to influenza-like illness in pregnancy with miscarriage, and neonatal disease. At greatest risk for disease are the fetus, neonates, cancer patients and immunocompromised persons. The organisms can grow at 4°C which means that organism **replication continues in refrigerated foods**.

Listeriosis has been categorized in two forms:

1) Adult disease

2) Neonatal disease

Adult Disease:

In normal healthy adults *Listeria* infections are generally asymptomatic or present as a mild flu-like illness. Some patients exhibit GI symptoms (watery diarrhea, fever, headache, myalgias, and abdominal cramps with little vomiting) Chills and fever are due to bacteremia.

In immunosuppressed individuals, however, it can produce serious illness, leading to meningitis. It is one of the leading causes of bacterial meningitis in patients with cancer and in renal transplant recipients. In the elderly, the early symptoms may go unnoticed and the infection may lead to acute manifestations of sepsis (high fever, hypo-tension). A complication of the bacteremia is endocarditis.

Neonatal Disease:

Neonatal disease can occur in **two forms**: disease acquired transplacentally *in utero* and disease acquired at birth or soon thereafter.

In utero acquired infection (**granulomatous infantiseptica**) causes abscesses and granulomas in multiple organs and very frequently results in abortion. This form of listeriosis has a high mortality rate unless promptly treated.

Post-delivery disease has 2 presentations, **early onset and late onset**. Early onset occurs within the first 5 days after exposure on vaginal delivery and is associated with sepsis and meningitis late onset disease occurs between 5 days to 3 weeks after delivery. Late onset presents with a **purulent meningitis or meningo-encephalitis with sepsis**.

Pathogenesis:

Upon infecting a cell (macrophages and parenchymal cells), the organism binds to the E-cadherin on the non-phagocytic cells via bacterial proteins (internalins). The bacteria are then taken up into phago-lysosomes. The low pH environment activates listeriolysin O, an exotoxin,

and **2 phospholipase C enzymes**. The bacteria are then released into the cytosol. Once in the cytoplasm the bacteria undergo rapid division and become encapsulated by short actin filaments. These filaments reorganize into a long tail extending from only one end of the bacterium. The tail mediates movement of the organism through the cytoplasm to the cell surface. At the cell periphery, protrusions (**filopods**) are formed that can penetrate neighboring cells and allow the bacterium to move into adjacent cells. Due to this mode of cell-cell transmission, the organisms are not exposed to the humoral anti-bacterial agents (*e.g.*, complement, antibody, *etc.*). *L. monocytogenes* is readily killed by activated macrophage.

Diagnosis:

Listeriosis is indicated when blood and CSF monocytosis is observed. The organism can be isolated on most laboratory media. Isolation of the organism from blood or spinal fluid is diagnostic.

Treatment and control:

Penicillin (ampicillin) alone or in combination with gentamycin has been effective. Immunity is cell-mediated.

ERYSIPELOID

Morphology and Physiology:

Erysipelothrix is a thin, pleomorphic, non-sporulating, non-motile, non-encapsulated, microaerophilic gram-positive rod that infects through skin abrasion while handling contaminated animal products or soil. Growth occurs on most commonly used laboratory media (*e.g.*, blood agar plates, chocolate agar plates). Optimal growth occurs at 30 to 37°C. After 24 hours at 37°C, colonies are small, circular, and transparent, with a smooth glistening surface and edge. Colonies may be of two types small and smooth or large and rough, developing after one to three days of incubation. A greening of the agar (α -hemolysis) under the colonies will develop after two days. The organism is catalase and oxidase negative.

Epidemiology and symptoms:

The organism is commensal or a pathogen in a large variety of vertebrate and invertebrate species. The major animal reservoir is domestic swine, but rodents, birds and fish are also frequently infected. Therefore, *Erysipelothrix rhusiopathiae* is considered an occupational disease with swine and fish handlers particularly at risk.

The organism gains entry into humans via scratches or puncture wounds of the skin. Three well-defined clinical categories of human disease include: (1) a localized cutaneous form, erysiploid; (2) a generalized cutaneous form; and (3) a septicemic form which is often associated with endocarditis. Erysipeloid is an inflammatory skin lesion (cellulitis), on fingers or hand. After 2 to 7 day incubation period lesions develop which are well-defined, slightly elevated, with a violaceous zone which spreads peripherally as discoloration in the central area fades. The pain is severe and may be described as a burning, throbbing, or itching sensation. It lacks suppuration and thus is distinguishable from staphylococcal erysipelas. The infection will resolve in 3 to 4 weeks without antibiotic treatment and sooner if antibiotics are administered. Systemic effects are uncommon.

The diffuse cutaneous form is rare. The cutaneous lesion progresses proximally

from the site of inoculation or appears in other areas. Blister formation may occur. The patients often have systemic manifestations such as fever and joint pains, but blood cultures are negative. The clinical course is more protracted, and recurrences are not uncommon. Systemic *E. rhusiopathiae* infections are infrequent.

Pathogenesis:

The virulence factors include hyaluronidase and neuraminidase.

Diagnosis:

The organism can be isolated from biopsy or tissue aspirates. The organism is not fastidious and can be grown in nutrient broth. Subculture on blood agar yields small α -hemolytic colonies.

Treatment and control: Erysipeloid is easily treatable with penicillin, but inherently resistant to vancomycin.

Bacillus

Two species of bacillus cause human disease, *B. anthracis* and *B. cereus*. These bacteria are aerobic, Gram-positive, spore forming, rods. They are present in soil and *B. cereus* is associated with the production of grains particularly rice. *B. anthracis* (anthrax) is considered an organism with bioterrorism potential.

ANTHRAX

Morphology and physiology:

Bacillus anthracis is the causative agent of anthrax. It is a Gram-positive, aerobic, spore-forming bacillus. Spores are formed in culture, in the soil, and in the tissues and exudates of dead animals, but **not** in the blood or internal tissues of living animals. Spores remain viable for extremely long periods of time (up to 50 years).

Epidemiology, transmission and symptoms:

Anthrax is a disease of herbivorous animals (cattle, sheep, horses, hogs, and goats). Humans are infected by direct contact (cutaneous) with contaminated material such as diseased animals, or working with hides, wool, or bone meal, by inhalation (**Woolsorter's disease**) of spores, or by ingestion of diseased animals. *B. anthracis* is not an invasive disease.

Cutaneous anthrax accounts for more than 95% of human cases. Spores enter through breaks in the skin, germinate and rapidly proliferate at the portal of entry. Within a few days, a small papule emerges that becomes vesicular. Rupture of this lesion will reveal a black eschar at the base surrounded by a zone of induration but no pus or pains are associated. This lesion is referred to as malignant pustule. The lesion is classically found on the hands, forearms, or head. The invasion of the bloodstream will lead to systemic dissemination of bacteria.

Pulmonary anthrax results from inhalation of *B. anthracis* spores which are phagocytized by the alveolar macrophages where they germinate and replicate. The organisms released from the dying cells and infect the hilar lymph node which leads to marked hemorrhagic necrosis. The patient may manifest fever, malaise, myalgia, and a nonproductive cough. Once in the hilar lymph node, infection may spread into the blood stream. Respiratory distress and

cyanosis are manifestations of toxemia. Death results within 24 hours. This form of anthrax is of significance in biological warfare.

Gastrointestinal anthrax: Ingestion of meat-derived from an infected animal leads to organism proliferation within the gastrointestinal tract, invasion of the epithelium, and ulceration of the mucosa. The organisms invade the mesenteric lymph nodes and disseminate into blood. Initially there is vomiting and diarrhea followed by blood in the feces. The invasion of the blood is associated with profound prostration, shock, and death. Because of strict control measures, this form of anthrax is not seen in the U.S.

Pathogenesis:

The virulence factors of *B. anthracis* include exotoxins and a capsule.

Exotoxin: A plasmid-encoded, protein complex made up of 3 components: 1) **Protective Antigen (PA)**, 2) **Edema Factor (EF)** and 3) **Lethal Factor (LF)**. *In vivo*, the PA combines with EF and LF to form 2 separate toxins.

The protective antigen functions as a ligand binding to surface receptor. Anthrax toxin receptor (ATR) is a type I membrane protein with an extracellular von Willebrand factor A domain. Once bound the PA is cleaved into two fragments by a cellular protease. The larger fragment then self-associates into ring-shaped heptamers. The heptamer binds up to three molecules of EF and/or LF. The complexes are endocytosed and trafficked to endosomes. The low pH induces conformational changes in the PA that allow it to form a membrane-spanning pore and translocates bound EF and/or LF across the membrane into the cytosol.

Edema Factor, when inside the cells binds to calmodulin and the complex acts as adenylate cyclase. EF causes a dramatic increase in cellular cAMP levels, upsetting water homeostasis and destroying the balance of intracellular signaling pathways. EF is responsible for the edema found in cutaneous anthrax.

Lethal factor is a zinc-dependent endopeptidase specific for two mitogen-activated protein kinase kinases (MAPKKs). PA plus LF inactivates MAPKKs inducing cell death of macrophages. Individually, the three proteins have no known toxic activity. Antibodies to protective antigens prevent PA binding to cells and stop EF and LF entry.

Capsule: It consists of a polypeptide of **D-glutamic acid** that is encoded by a plasmid and is antiphagocytic. It is not a good immunogen and even if any antibodies are produced, they are not protective against the disease.

Diagnosis:

Clinical diagnosis of anthrax can be confirmed by direct examination or culture. Fresh smears of vesicular fluid, fluid from under the eschar, blood, or spleen or lymph node aspirates. Cultured organism stains as Gram-positive long thin rods.

Prevention and Treatment:

Most *B. anthracis* strains are sensitive to a broad range of antibiotics (Penicillin, ciprofloxacin, or doxycycline). Quarantine is not needed. Exposure to anthrax requires antibiotic treatment for 60 days. Currently one human vaccine is available.

Bacillus cereus

Morphology and physiology:

B. cereus is a close relative to *B. anthracis*. It is a Gram-positive, aerobic, spore-forming bacillus found in soil. It causes food poisoning, and eye infections such as severe keratitis, endophthalmitis, and panophthalmitis. In contrast to colonies of *B. anthracis*, *B. cereus* colonies are β -hemolytic.

Symptoms:

Two types of food poisoning are associated with *B. cereus*: Emetic which causes, nausea, vomiting, abdominal cramps. This form of food poisoning has an incubation period 1-5 hours and is self limiting with recovery within 24 hours. The second form is diarrheal with an incubation period 1-24 hours. Diarrheal food poisoning exhibits profuse diarrhea with abdominal pain and cramps, but fever and vomiting are uncommon.

The eye infections are generally seen after penetrating trauma. Symptoms depend on the type of trauma.

Pathogenesis:

Three enterotoxins have been identified, hemolytic, non-hemolytic and a cytotoxin, in addition to cereulide, a heat-stable emetic toxin.