CHAPTER

20

Gram-Negative Rods Related to Animal Sources (Zoonotic Organisms)

CHAPTER CONTENTS

INTRODUCTION

Zoonoses are human diseases caused by organisms that are acquired from animals. There are bacterial, viral, fungal, and parasitic zoonoses. Some zoonotic organisms are acquired directly from the animal reservoir, whereas others are transmitted by vectors, such as mosquitoes, fleas, or ticks.

There are four medically important gram-negative rods that have significant animal reservoirs: *Brucella* species, *Francisella tularensis, Yersinia pestis*, and *Pasteurella multocida* (Table 20–1).

Additional information regarding the clinical aspects of infections caused by the organisms in this chapter is provided in Part IX entitled Infectious Diseases beginning on page 593.

BRUCELLA

Disease

Brucella species cause brucellosis (undulant fever).

Important Properties

Brucellae are small gram-negative rods without a capsule. The three major human pathogens and their animal reservoirs are *Brucella melitensis* (goats and sheep), *Brucella abortus* (cattle), and *Brucella suis* (pigs).

Pathogenesis & Epidemiology

The organisms enter the body either by ingestion of **contaminated milk products** or **through the skin** by direct

TABLE 20–1 Gram-Negative Rods Associated with Animal Sources

Species	Disease	Source of Human Inflection	Mode of Transmission from Animal to Human	Diagnosis
Brucella species	Brucellosis	Pigs, cattle, goats, sheep	Dairy products; contact with animal tissues	Serology or culture
Francisella tularensis	Tularemia	Rabbits, deer, ticks	Contact with animal tissues; ticks	Serology
Yersinia pestis	Plague	Rodents	Flea bite	Immunofluorescence or culture
Pasteurella multocida	Cellulitis	Cats, dogs	Cat or dog bite	Wound culture
Bartonella henselae	Cat-scratch disease and bacillary angiomatosis	Cats	Cat scratch or bite; bite of cat flea	Serology or Warthin-Starry silver stain of tissue

contact in an occupational setting such as an abattoir. They localize in the **reticuloendothelial system,** namely, the lymph nodes, liver, spleen, and bone marrow. Many organisms are killed by macrophages, but some survive within these cells, where they are protected from antibody. The host response is granulomatous, with lymphocytes and epithelioid giant cells, which can progress to form focal abscesses. The mechanism of pathogenesis of these organisms is not well defined, except that endotoxin is involved. No exotoxins are produced.

Imported cheese made from unpasteurized goats' milk produced in either Mexico or the Mediterranean region has been a source of *B. melitensis* infection in the United States. The disease occurs worldwide but is rare in the United States because pasteurization of milk kills the organism.

Clinical Findings

After an incubation period of 1 to 3 weeks, nonspecific symptoms such as fever, chills, fatigue, malaise, anorexia, and weight loss occur. The onset can be acute or gradual. The undulating (rising-and-falling) fever pattern that gives the disease its name occurs in a minority of patients. Enlarged lymph nodes, liver, and spleen are frequently found. Pancytopenia occurs. *Brucella melitensis* infections tend to be more severe and prolonged, whereas those caused by *B. abortus* are more self-limited. Osteomyelitis is the most frequent complication. Secondary spread from person to person is rare.

Laboratory Diagnosis

Recovery of the organism requires the use of enriched culture media and incubation in 10% CO_2. The organisms can be presumptively identified by using a slide agglutination test with *Brucella* antiserum, and the species can be identified by biochemical tests. If organisms are not isolated, analysis of a serum sample from the patient for a rise in antibody titer to *Brucella* can be used to make a diagnosis. In the absence of an acute-phase serum specimen, a titer of at least 1:160 in the convalescent-phase serum sample is diagnostic.

Treatment

The treatment of choice is tetracycline plus rifampin. There is no significant resistance to these drugs.

Prevention

Prevention of brucellosis involves pasteurization of milk, immunization of animals, and slaughtering of infected animals. There is no human vaccine.

FRANCISELLA

Disease

Francisella tularensis causes tularemia.

Important Properties

Francisella tularensis is a small, pleomorphic gram-negative rod. It has a single serologic type. There are two biotypes, A and B, which are distinguished primarily on their virulence and epidemiology. Type A is more virulent and found primarily in the United States, whereas type B is less virulent and found primarily in Europe.

Pathogenesis & Epidemiology

Francisella tularensis is remarkable in the wide variety of animals that it infects and in the breadth of its distribution in the United States. It is enzootic (endemic in animals) in every state, but most human cases occur in the rural areas of Arkansas and Missouri. It has been isolated from more than 100 different species of **wild animals,** the most important of which are rabbits, deer, and a variety of rodents. The bacteria are transmitted among these animals by vectors such as **ticks,** mites, and lice, especially the *Dermacentor* ticks that feed on the blood of wild rabbits. The tick maintains the chain of transmission by passing the bacteria to its offspring by the transovarian route. In this process, the bacteria are passed through ovum, larva, and nymph stages to adult ticks capable of transmitting the infection.

Humans are accidental "dead-end" hosts who acquire the infection most often by being bitten by the vector or by having skin contact with the animal during removal of the hide. Rarely, the organism is ingested in infected meat, causing gastrointestinal tularemia, or is inhaled, causing pneumonia. There is no person-to-person spread. The main type of tularemia in the United States is tick-borne tularemia from a rabbit reservoir.

The organism enters through the skin, forming an ulcer at the site in most cases. It then localizes to the cells of the reticuloendothelial system, and granulomas are formed. Caseation necrosis and abscesses can also occur. Symptoms are caused primarily by endotoxin. No exotoxins have been identified.

Clinical Findings

Presentation can vary from sudden onset of an influenza-like syndrome to prolonged onset of a low-grade fever and adenopathy. Approximately 75% of cases are the "ulceroglandular" type, in which the site of entry ulcerates and the regional lymph nodes are swollen and painful. Other, less frequent forms of tularemia include glandular, oculoglandular, typhoidal, gastrointestinal, and pulmonary. Disease usually confers lifelong immunity.

Laboratory Diagnosis

Attempts to culture the organism in the laboratory are rarely undertaken, because there is a high risk to laboratory workers of infection by inhalation, and the special cysteine-containing medium required for growth is not usually available. The most frequently used diagnostic method is

the agglutination test with acute- and convalescent-phase serum samples. Fluorescent-antibody staining of infected tissue can be used if available.

Treatment

Streptomycin is the drug of choice. There is no significant antibiotic resistance.

Prevention

Prevention involves avoiding both being bitten by ticks and handling wild animals. There is a live, attenuated bacterial vaccine that is given only to persons, such as fur trappers, whose occupation brings them into close contact with wild animals. The vaccine is experimental and not available commercially but can be obtained from the U.S. Army Medical Research Command, Fort Detrick, Maryland. This and the bacillus of Calmette-Guérin (BCG) vaccine for tuberculosis are the only two live bacterial vaccines for human use.

YERSINIA

Disease

Yersinia pestis is the cause of plague, also known as the black death, the scourge of the Middle Ages. It is also a contemporary disease, occurring in the western United States and in many other countries around the world. Two less important species, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, are described in Chapter 27.

Important Properties

Yersinia pestis is a small gram-negative rod that exhibits bipolar staining (i.e., it **resembles a safety pin**, with a central clear area). Freshly isolated organisms possess a capsule composed of a polysaccharide–protein complex. The capsule can be lost with passage in the laboratory; loss of the capsule is accompanied by a loss of virulence. It is one of the **most virulent** bacteria known and has a strikingly low ID_{50} (i.e., 1–10 organisms are capable of causing disease).

Pathogenesis & Epidemiology

The plague bacillus has been endemic in the wild rodents of Europe and Asia for thousands of years but entered North America in the early 1900s, probably carried by a rat that jumped ship at a California port. It is now endemic in the wild rodents in the western United States, although 99% of cases of plague occur in Southeast Asia.

The enzootic (sylvatic) cycle consists of transmission among **wild rodents by fleas.** In the United States, prairie dogs are the main reservoir. Rodents are relatively resistant to disease; most are asymptomatic. Humans are accidental hosts, and cases of plague in this country occur as a result of being bitten by a flea that is part of the sylvatic cycle.

The urban cycle, which does not occur in the United States, consists of transmission of the bacteria among urban rats (the reservoir), with the **rat flea** as vector. This cycle predominates during times of poor sanitation (e.g., wartime), when rats proliferate and come in contact with the fleas in the sylvatic cycle.

The events within the flea are fascinating as well as essential. The flea ingests the bacteria while taking a blood meal from a bacteremic rodent. A thick biofilm containing many organisms forms in the upper gastrointestinal tract that prevents any food from proceeding down the gastrointestinal tract of the flea. This "blocked flea" then regurgitates the organisms into the bloodstream of the next animal or human it bites.

The organisms inoculated at the time of the bite spread to the regional lymph nodes, which become swollen and tender. These swollen lymph nodes are the **buboes** that have led to the name **bubonic plague.** The organisms can reach high concentrations in the blood (bacteremia) and disseminate to form abscesses in many organs. The **endotoxin-related symptoms,** including disseminated intravascular coagulation and cutaneous hemorrhages, probably were the genesis of the term **black death.**

In addition to the sylvatic and urban cycles of transmission, respiratory droplet transmission of the organism from patients with pneumonic plague can occur.

The organism has several factors that contribute to its virulence: (1) the envelope capsular antigen, called F-1, which protects against phagocytosis; (2) endotoxin; (3) an exotoxin; and (4) two proteins known as V antigen and W antigen. The V and W antigens allow the organism to survive and grow intracellularly, but their mode of action is unknown. The action of the exotoxin is unknown.

Other factors that contribute to the extraordinary pathogenicity of *Y. pestis* are a group of virulence factors collectively called **Yops** (*Yersinia* **outer proteins**). These are injected into the human cell via type III secretion systems and inhibit phagocytosis and cytokine production by macrophages and neutrophils. For example, one of the Yops proteins (YopJ) is a protease that cleaves two signal transduction pathway proteins required for the induction of tumor necrosis factor synthesis. This inhibits the activation of our host defenses and contributes to the ability of the organism to replicate rapidly within the infected individual.

Clinical Findings

Bubonic plague, which is the most frequent form, begins with pain and swelling of the lymph nodes draining the site of the flea bite and systemic symptoms such as high fever, myalgias, and prostration. The affected nodes enlarge and become exquisitely tender. These buboes are an early characteristic finding. Septic shock and pneumonia are the main life-threatening subsequent events. Pneumonic plague can arise either from inhalation of an aerosol or from septic emboli that reach the lungs. Untreated bubonic

plague is fatal in approximately half of the cases, and untreated pneumonic plague is invariably fatal.

Laboratory Diagnosis

Smear and culture of blood or pus from the bubo is the best diagnostic procedure. Great care must be taken by the physician during aspiration of the pus and by laboratory workers doing the culture not to create an aerosol that might transmit the infection. Giemsa or Wayson stain reveals the typical safety-pin appearance of the organism better than does Gram stain. Fluorescent-antibody staining can be used to identify the organism in tissues. A rise in antibody titer to the envelope antigen can be useful retrospectively.

Treatment

The treatment of choice is a combination of streptomycin and a tetracycline such as doxycycline, although streptomycin alone can be used. Levofloxacin can also be used. There is no significant antibiotic resistance. In view of the rapid progression of the disease, treatment should not wait for the results of the bacteriologic culture. Incision and drainage of the buboes are not usually necessary.

Prevention

Prevention of plague involves controlling the spread of rats in urban areas, preventing rats from entering the country by ship or airplane, and avoiding both flea bites and contact with dead wild rodents. A patient with plague must be placed in strict isolation (quarantine) for 72 hours after antibiotic therapy is started. Only close contacts need to receive prophylactic tetracycline, but all contacts should be observed for fever. Reporting a case of plague to the public health authorities is mandatory.

A vaccine consisting of formalin-killed organisms provides partial protection against bubonic but not pneumonic plague. This vaccine was used in the armed forces during the Vietnam War but is not recommended for tourists traveling to Southeast Asia.

PASTEURELLA

Disease

Pasteurella multocida causes wound infections associated with cat and dog bites.

Important Properties

Pasteurella multocida is a short, encapsulated gram-negative rod that exhibits bipolar staining.

Pathogenesis & Epidemiology

The organism is part of the normal flora in the mouths of many animals, particularly **domestic cats and dogs,** and is transmitted by **biting.** About 25% of animal bites become infected with the organism, with sutures acting as a predisposing factor to infection. Most bite infections are polymicrobial, with a variety of facultative anaerobes, especially *Streptococcus* species, and anaerobic organisms present in addition to *P. multocida*. Pathogenesis is not well understood, except that the capsule is a virulence factor and endotoxin is present in the cell wall. No exotoxins are made.

Clinical Findings

A rapidly spreading cellulitis at the site of an animal bite is indicative of *P. multocida* infection. The incubation period is brief, usually less than 24 hours. Osteomyelitis can complicate cat bites in particular, because cats' sharp, pointed teeth can implant the organism under the periosteum.

Laboratory Diagnosis

The diagnosis is made by finding the organism in a culture of a sample from the wound site.

Treatment

Penicillin G is the treatment of choice. There is no significant antibiotic resistance.

Prevention

People who have been bitten by a cat should be given ampicillin to prevent *P. multocida* infection. Animal bites, especially cat bites, should not be sutured.

BARTONELLA

Disease

Bartonella henselae is the cause of cat-scratch disease and bacillary angiomatosis. Cat-scratch disease is one of the most common zoonotic diseases in the United States.

Important Properties

Bartonella henselae is a small, pleomorphic gram-negative rod. It is a fastidious organism and will not grow on routine blood agar. It can be cultured on specialized media in the clinical laboratory.

Pathogenesis & Epidemiology

Cat scratches or bites, especially from kittens, are the main mode of transmission of *B. henselae* to humans. The organism is a member of the oral flora of many cats. There is evidence that it is transmitted from cats to humans by the bite of cat fleas. Exposure to cat urine or feces does not pose a risk of transmission. Person-to-person transmission of *B. henselae* does not play a significant role in infection. *Bartonella henselae* is a low virulence organism, and disease is self-limited in immunocompetent individuals.

The pathogenesis of angiomas that occur in *Bartonella* infections in immunocompromised individuals is uncertain. One current explanation is that infection of endothelial cells by *Bartonella* induces the synthesis of angiogenesis factor that causes endothelial cells to proliferate.

Clinical Findings

In immunocompetent people, *B. henselae* causes **cat-scratch disease (CSD).** This disease is characterized by fever and tender, enlarged lymph nodes, typically on the same side as the scratch (Figure 20–1). A papule at the site of the scratch may precede the lymphadenopathy. CSD has a prolonged course but eventually resolves, even without antibiotics. A small percentage of those infected develop systemic disease, such as endocarditis or encephalitis.

In immunocompromised individuals, especially patients with acquired immunodeficiency syndrome (AIDS), *B. henselae* causes bacillary angiomatosis (BA). BA is characterized by raised, cherry-red vascular lesions in the skin and visceral organs (Figure 20–2). The lesions appear papular or nodular. Bacillary peliosis (peliosis hepatis) is similar to bacillary angiomatosis except that in peliosis, the lesions occur primarily in the liver and spleen.

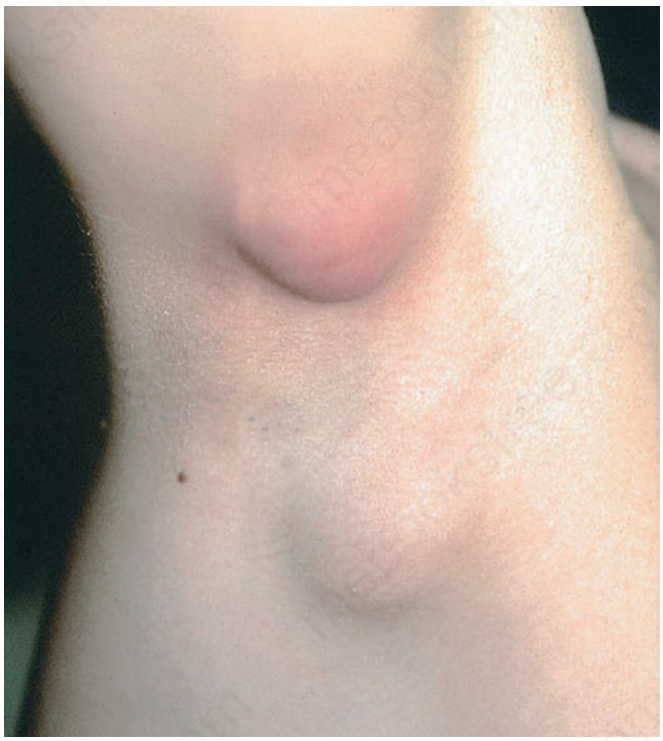

FIGURE 20–1 Cat-scratch disease. Note the two enlarged, inflamed axillary lymph nodes in a patient with cat-scratch disease. (Reproduced with permission from Wolff K, Johnson R. *Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology*. 6th ed. New York: McGraw-Hill, 2009. Copyright © 2009 by The McGraw-Hill Companies, Inc.)

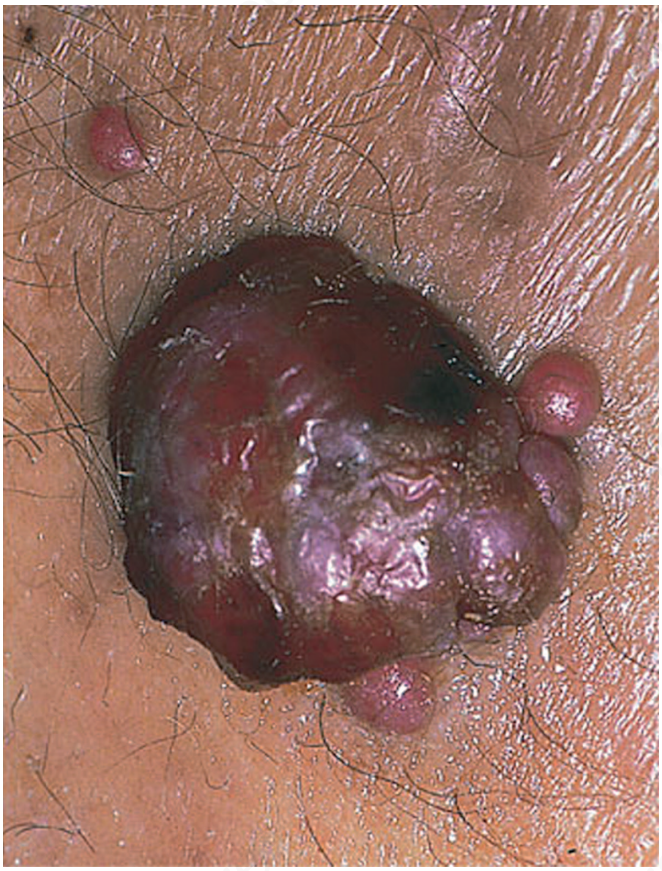

FIGURE 20–2 Bacillary angiomatosis. Note the cherry-red hemangioma-like skin lesion. (Reproduced with permission from Wolff K, Johnson R. *Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology*. 6th ed. New York: McGraw-Hill, 2009. Copyright © 2009 by The McGraw-Hill Companies, Inc.)

Laboratory Diagnosis

The diagnosis of CSD is usually made serologically. Antibodies against *B. henselae* antigens can be detected in a patient's serum by a variety of immunologic tests. The organism can be cultured on artificial media but takes 5 days or longer to grow and so is not usually done. The diagnosis of BA is often made by finding pleomorphic rods in biopsy tissue using the Warthin-Starry silver stain. Pathologic examination of tissue from the lesion will distinguish bacillary angiomatosis from Kaposi's sarcoma.

Treatment

No antibiotic therapy is typically recommended for CSD. If the patient has severe lymphadenitis, azithromycin is the drug of choice. Treatment of BA with doxycycline or erythromycin is effective. There is no significant antibiotic resistance.

Prevention

Antibiotics are not recommended for people who have sustained a cat scratch. There is no vaccine.

SELF-ASSESSMENT QUESTIONS

1. Your patient is a 10-year-old boy who has a high fever and swollen, painful axillary lymph nodes on the left side. His mother says that he brought home a dead rat a few days ago. You suspect he may have bubonic plague. Regarding the causative organism, which one of the following is most accurate?

(A) It has a very low ID_{50}.
(B) It is transmitted from rodents to humans by ticks.
(C) It is endemic primarily in the states along the East Coast of the United States.
(D) Its main virulence factor is an exotoxin that induces interleukin-2 (IL-2) production by CD4-positive helper T cells.
(E) Infection should be treated with high doses of penicillin G intravenously.

2. Your patient is a 20-year-old man who was bitten on the hand when he tried to break up a fight between two cats yesterday. He now has a red, hot, tender, swollen lesion at the bite site that has spread rapidly across his hand. Which one of the following bacteria is the most likely cause of his cellulitis?

(A) *Brucella melitensis*
(B) *Francisella tularensis*
(C) *Pasteurella multocida*
(D) *Yersinia pestis*

3. Your patient is a 30-year-old woman who reports that she has had intermittent fever of 102°F, sweating, and fatigue for the past month or so. She has lost her appetite and has lost about 10 pounds in that period. She enjoys eating unpasteurized goat cheese. On examination, hepatosplenomegaly is detected. A blood count reveals pancytopenia. Which one of the following bacteria is the most likely cause of this infection?

(A) *Brucella melitensis*
(B) *Francisella tularensis*
(C) *Pasteurella multocida*
(D) *Yersinia pestis*

4. Regarding *B. henselae*, which one of the following is most accurate?

(A) *Bartonella henselae* is an anaerobic, spore-forming, gram-positive rod.
(B) The natural habitat of *B. henselae* is the cat's mouth.
(C) *Bartonella henselae* causes cellulitis in immunocompromised patients such as AIDS patients.
(D) Diagnosis in the clinical laboratory depends on detecting antibodies in the patient's serum that will agglutinate cardiolipin.
(E) The drug of choice for *B. henselae* infections is metronidazole.

ANSWERS

1. **(A)**
2. **(C)**
3. **(A)**
4. **(B)**

SUMMARIES OF ORGANISMS

Brief summaries of the organisms described in this chapter begin on page 663. Please consult these summaries for a rapid review of the essential material.

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Clinical Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 713. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.