The Brucellae, Yersinia and Leptospira

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The brucellae are obligate parasites of animals and humans and are characteristically located intracellularly.

They are relatively inactive metabolically. Brucella melitensis typically infects goats; Brucella suis, swine; Brucella abortus, cattle; and Brucella canis, dogs. Other species are found only in animals.

Although named as species, DNA relatedness studies have shown there is only one species in the genus, B melitensis, with multiple biovars.

The disease in humans, brucellosis (undulant fever, Malta fever), is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.
Morphology and Identification

- The appearance in young cultures varies from cocci to rods 1.2 μm in length, with short coccobacillary forms predominating. They are gram negative but often stain irregularly, and they are aerobic, non-motile, and non-spore forming.

- Brucellae are adapted to an intracellular habitat, and their nutritional requirements are complex.

- Whereas B abortus requires 5–10% CO2 and Cystine for growth, the other three species grow in air.

- Catalase and oxidase are produced by the four species that infect humans.

- Brucellae are adapted to an intracellular habitat.

- They are killed by boiling and pasteurization but are resistant to freezing and drying.
Epidemiology

• Brucellae are animal pathogens transmitted to humans by accidental contact with infected animal feces, urine, milk, or tissues. The common sources of infection for humans are unpasteurized milk, milk products, and cheese as well as occupational contact (e.g., farmers, veterinarians, and slaughterhouse workers) with infected animals. Cheese made from unpasteurized goat’s milk is a particularly common vehicle for transmission of brucellosis.

• Brucellosis may be acquired by ingestion, inhalation, or mucosal or percutaneous exposure.

• Accidental injection of the live vaccine strains of B. abortus (S19 and RB51) and B. melitensis (Rev 1) can cause disease. B. melitensis and B. suis have historically been developed as biological weapons by several countries and could be exploited for bioterrorism.
Antigenic Structure

• Differentiation among Brucella species or biovars is made possible by their characteristic sensitivity to dyes and their production of H2S.

• Few laboratories have maintained the procedures for these tests, and the brucellae are seldom placed into the traditional species. Because brucellae are hazardous in the laboratory, tests to classify them should be performed only in reference public health laboratories using appropriate biosafety precautions.
Pathogenesis

• Although each species of Brucella has a preferred host, all can infect a wide range of animals, including humans.

• The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurized goats’ milk is a particularly common vehicle.

• The organisms progress from the portal of entry via lymphatic channels and regional lymph nodes to the thoracic duct and the bloodstream, which distributes them to the parenchymatous organs. Granulomatous nodules that may develop into abscesses form in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system. In such lesions, the brucellae are principally intracellular.

• Osteomyelitis, meningitis, or cholecystitis also occasionally occurs. The main histologic reaction in brucellosis consists of proliferation of mononuclear cells, exudation of fibrin, coagulation necrosis, and fibrosis.

• The granulomas form and consist of epithelioid and giant cells, with central necrosis and peripheral fibrosis.
Clinical Findings

• The incubation period ranges from 1–4 weeks. The onset is insidious, with malaise, fever, weakness, aches, and sweats.

• The fever usually rises in the afternoon; its fall during the night is accompanied by drenching sweat.

• There may be gastrointestinal and nervous symptoms. Lymph nodes enlarge, and the spleen becomes palpable. Hepatitis may be accompanied by jaundice.

• Deep pain and disturbances of motion, particularly in vertebral bodies, suggest osteomyelitis. These symptoms of generalized Brucella infection generally subside in weeks or months, although localized lesions and symptoms may continue.

• After the initial infection, a chronic stage may develop, characterized by weakness, aches and pains, low-grade fever, nervousness, and other nonspecific manifestations compatible with psychoneurotic symptoms.
Diagnostic Laboratory Tests

A. Specimens
- Blood should be taken for culture, biopsy material for culture (lymph nodes, bone, and so on), and serum for serologic tests.

B. Culture
- Brucella agar, specifically designed to culture Brucella species bacteria. The medium is highly enriched and—in reduced form—is used primarily in cultures for anaerobic bacteria.
- Brucella species bacteria grow on commonly used media, including trypticase-soy medium with or without 5% sheep blood, brain–heart infusion medium, and chocolate agar.
- The typical virulent organism forms a smooth, transparent colony; upon culture
C. Serology

- Immunoglobulin M (IgM) antibody levels rise during the first week of acute illness, peak at 3 months, IgG and IgA antibody levels rise about 3 weeks after onset of acute disease, peak at 6–8 weeks, and remain high during chronic disease.

- Agglutination test: IgG agglutinin titers above 1:80 indicate active infection. Individuals injected with cholera vaccine may develop agglutination titers to brucellae.

- Blocking antibodies— These are IgA antibodies that interfere with agglutination by IgG and IgM and cause a serologic test result to be negative in low serum dilutions (prozone), although positive in higher dilutions. These antibodies appear during the subacute stage of infection, tend to persist for many years independently of activity of infection, and are detected by the Coombs antiglobulin method.

- ELISA assays— IgG, IgA, and IgM antibodies may be detected using enzyme-linked immunosorbent assay (ELISA), which use cytoplasmic proteins as antigens. These assays tend to be more sensitive and specific than the agglutination test especially in the setting of chronic disease.
Treatment & Immunity

• Brucellae may be susceptible to tetracyclines, rifampin, trimethoprim–sulfamethoxazole, aminoglycosides, and some quinolones. Symptomatic relief may occur within a few days after treatment with these drugs. However, because of their intracellular location, the organisms are not readily eradicated completely from the host.

• For best results, treatment must be prolonged. Combined treatment with a tetracycline (eg, doxycycline) and either streptomycin for 2–3 weeks or rifampin for 6 weeks is recommended.
Prevention, and Control

• Eradication of brucellosis in cattle can be attempted by test and slaughter, active immunization of heifers with avirulent live strain 19, or combined testing, segregation, and immunization. Cattle are examined by means of agglutination tests.

• Active immunization of humans against Brucella infection is experimental.

• Control rests on limitation of spread and possible eradication of animal infection, pasteurization of milk and milk products, and reduction of occupational hazards wherever possible.
Yersinia

• The genus Yersinia comprises gram-negative bacteria of the family Enterobacteriaceae (gamma proteobacteria).
• They grow best at 25°C and are motile at 25°C but nonmotile at 37°C.
• Y pestis –plague–is transmitted to humans usually through the bite of an infected flea, although inhalation is another potential route.
• Yersiniosis is a zoonotic infection with an enteropathogenic Yersinia species, usually Yersinia enterocolitica or Y. pseudotuberculosis.
• Y. enterocolitica is more closely associated with terminal ileitis and Y. pseudotuberculosis with mesenteric adenitis, but both organisms may cause mesenteric adenitis and symptoms of abdominal pain and tenderness that result in pseudoappendicitis, with the surgical removal of a normal appendix.
• Y. enterocolitica is found worldwide and has been isolated from a wide variety of wild and domestic animals and environmental samples, including samples of food and water.

• Most clinical infections are associated with serogroups O:3, O:9, and O:5,27, with a declining number of O:8 infections.

• Consumption or preparation of raw meat, products milk (pasteurized, unpasteurized, and chocolate-flavored) and various foods contaminated with spring water products are linked with infection.

• Y. pseudotuberculosis is less frequently reported as a cause of human disease than Y. enterocolitica.
Pathogenesis

- The usual route of infection is oral. Initial replication in the small intestine is followed by invasion of Peyer’s patches of the distal ileum via M cells, with onward spread to mesenteric lymph nodes. The liver and spleen can also be involved after oral infection.

- The characteristic histologic appearance of enteropathogenic yersiniae after invasion of host tissues is as extracellular micro-abscesses surrounded by an epithelioid granulomatous lesion.

- *Y. enterocolitica* can produce a heat-stable enterotoxin, but the role of this toxin in diarrhea associated with infection is not well defined.

- All *yersinia* possess lipopolysaccharides that have endotoxic activity when released.

- They have type III secretion systems that consist of a membrane-spanning complex that allows the bacteria to inject proteins directly into cytoplasm of the host cells.

- The pathogenic *yersinia* have a pathogenicity island (PAI) that encodes for an iron-scavenging siderophore.
Clinical manifestations

• Self-limiting diarrhea is the most common reported presentation in infection with pathogenic Y. enterocolitica, especially in children under the age of 4, who form the single largest group in most case series.

• Blood may be detected in diarrheal stool. Older children and adults are more likely than younger children to present with abdominal pain, which can be localized to the right iliac fossa—a situation that often leads to laparotomy for presumed appendicitis (pseudoappendicitis).

• Gastrointestinal complications include granulomatous appendicitis, a chronic inflammatory condition affecting the appendix.

• Post-infective phenomena of reactive arthritis might be developing within 2–4 weeks of a preceding infection.
LABORATORY DIAGNOSIS

- **Specimens**
  - Specimens may be stool, blood, or material obtained at surgical exploration.

- **Culture**
  - The number of yersiniae in stool may be small and can be increased by “cold enrichment”. Subcultures made at intervals on MacConkey agar may yield yersiniae. Alternatively, most clinical laboratories use a Yersinia selective agar such as cefsulodin-Irgasan-novobiocin (CIN) agar incubated at room temperature for several days.
  - *Y enterocolitica* colonies have a bull’s eye appearance with a red center on CIN agar.

- **Serology**
  - Serum specimens taken 2 or more weeks apart, a rise in agglutinating antibodies can be shown; however, cross-reactions between yersiniae and other organisms (vibrios, salmonellae, and brucellae) may confuse the results.
Treatment

• Most cases of diarrhea caused by enteropathogenic Yersinia are self-limiting. Data from clinical trials do not support antimicrobial treatment for adults or children with Y. enterocolitica diarrhea.

• Systemic infections with bacteremia or focal infections outside the gastrointestinal tract generally require antimicrobial therapy.

• Fluoroquinolone therapy is effective for bacteremia in adults; for example, ciprofloxacin, A third-generation cephalosporin is an alternative
PREVENTION AND CONTROL

• Safe handling and processing of food.

• No vaccine is effective in preventing intestinal colonization of food animals by enteropathogenic Yersinia.

• Consumption of food made from raw meat should be discouraged at present because it is not possible to eliminate contamination with the enteropathogenic Yersinia strains found worldwide.
Leptospira

• Traditionally, the genus Leptospira comprised two species: the pathogenic L. interrogans and the free-living L. biflexa, now designated L. interrogans sensu lato and L. biflexa sensu lato, respectively.

• Leptospirosis; The disease is caused by pathogenic Leptospira species and is characterized by a broad spectrum of clinical manifestations, varying from asymptomatic infection to fulminant, fatal disease (Weil’s Syndrome).

• Kidney involvement in many animal species is chronic and results in the shedding of large numbers of leptospiroæ in the urine; this is probably the main source of environmental contamination resulting in infection of humans.

• Human urine also may contain spirochetes in the second and third weeks of disease.
Leptospira interrogans

• Leptospirae are tightly coiled, thin, flexible spirochetes 5–15 μm long, with very fine spirals 0.1–0.2 μm wide; one end is often bent, forming a hook. They are motile.

• They are actively motile, which is best seen using a dark-field microscope.

• Leptospirae derive energy from oxidation of long-chain fatty acids and cannot use amino acids or carbohydrates as major energy sources. Ammonium salts are a main source of nitrogen.

• Leptospirae can survive for weeks in water, particularly at alkaline pH.
Epidemiology

• Leptospirosis has a worldwide distribution but occurs most commonly in the tropics and subtropics because the climate and occasionally poor hygienic conditions favor the pathogen’s survival and distribution.

• Current information on global human leptospirosis varies but indicates that approximately 1 million severe cases occur per year, with a mean case–fatality rate of nearly 10%.

• The vast majority of infections with Leptospira cause no or only mild disease in humans. A small percentage of infections (~1%) lead to severe, potentially fatal complications.
Antigenic Structure

• The outer envelope contains large amounts of lipopolysaccharide of antigenic structure that is variable from one strain to another. This variation forms the basis for the serologic classification of the *Leptospira* species.

• *L. Grippotyphosa*, *L. Mitis*, *L. Canicola*, *L. Icterohaemorrhagiae* are among the serogroups that are Principal cause of Leptospiral Diseases
Pathogenesis

• Transmission occurs through cuts, abraded skin, or mucous membranes, especially the conjunctival and oral mucosa. After entry, and an incubation period of 1–2 weeks the organisms proliferate, cross tissue barriers, and disseminate hematogenously to all organs (leptospiremic phase).

• They then establish themselves in the parenchymatous organs (particularly liver and kidneys), producing hemorrhage and necrosis of tissue and resulting in dysfunction of those organs (jaundice, hemorrhage, nitrogen retention).
Clinical Findings

• The illness is often biphasic. After initial improvement, the second phase develops when the IgM antibody titer rises. It manifests itself often as “aseptic meningitis” with an intense headache, stiff neck, and pleocytosis of the CSF.

• Nephritis and hepatitis may also recur, and there may be skin, muscle, and eye lesions. The degree and distribution of organ involvement vary in the different diseases produced by different leptospiroae in various parts of the world.

• Human urine also may contain spirochetes in the second and third weeks of disease.

• Many infections are mild or subclinical. Hepatitis is frequent in patients with leptospirosis.
Diagnostic Laboratory Tests

• A. Specimens
• Specimens consist of blood, CSF, or urine and tissues for microscopic examination and culture.

• B. Microscopic Examination
• Dark-field examination or thick smears stained by the Giemsa technique.

• C. Culture
• Whole fresh blood, CSF or urine or crushed tissue can be cultured. Leptospires grow best under aerobic conditions at 28–30 C in semisolid medium (eg, Ellinghausen-McCullough-Johnson- Harris EMJH) in 10 mL test tubes with 0.1% agar and 5-fluorouracil.
• Growth is slow, and cultures should be kept for at least 8 weeks.

• D. Serology
• The diagnosis of leptospirosis in most cases is confirmed serologically with microscopic agglutination test (MAT) and ELISA.
Treatment & Immunity

- Treatment of mild leptospirosis should be with oral doxycycline, ampicillin, or amoxicillin.

- Severe leptospirosis should be treated with IV penicillin as soon as the diagnosis is considered.

- Serovar-specific immunity follows infection, but reinfection with different serovars may occur.
Prevention, and Control

• Leptospirosis is excreted in urine both during the active illness and during the asymptomatic carrier state.

• Leptospirosis remain viable in stagnant water for several weeks; drinking, swimming, bathing, or food contamination may lead to human infection. Persons most likely to come in contact with water contaminated by rats (e.g., miners, sewer workers, farmers, and fishermen) run the greatest risk of infection.

• Avoidance of exposure to urine and tissues from infected animals through proper eyewear, footwear, and other protective equipment. Targeted rodent control strategies could be considered.

• Vaccines for agricultural and companion animals are generally available, and their use should be encouraged.
The End