

The Brucellae, Leptospira and Mycobacterium of the GIT

By : Nader Alaridah MD, PhD

THE BRUCELLAE

- 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, nonmotile, and nonspore forming.
- Brucellae are adapted to an intracellular habitat, and their nutritional requirements are complex.
- Whereas *B abortus* requires 5–10% CO_2 for growth, the other three species grow in air.
- Catalase and oxidase are produced by the four species that infect humans.
- 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 (eg, 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.

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.
- ❑ 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

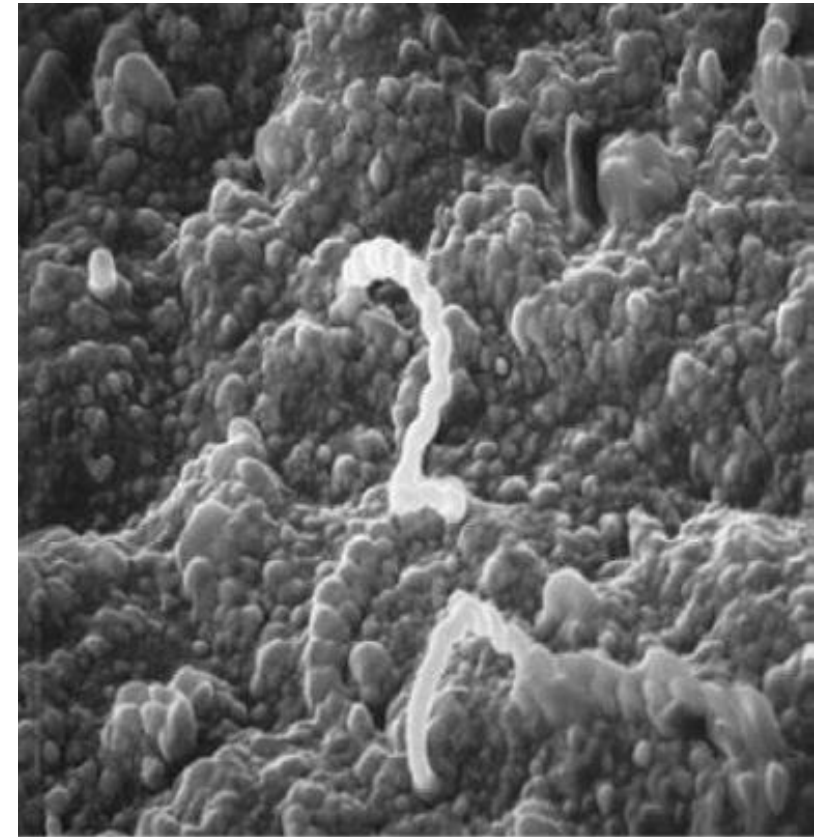
- Eradication of brucellosis in cattle can be attempted by test and slaughter, active immunization of heifers with avirulent live strain 19, or combined testing, segregation, and immunization. Cattle are examined by means of agglutination tests.
- Active immunization of humans against *Brucella* infection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

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 leptospirae 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.



0.3 μm

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.

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 leptospirae 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. Leptospire 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.

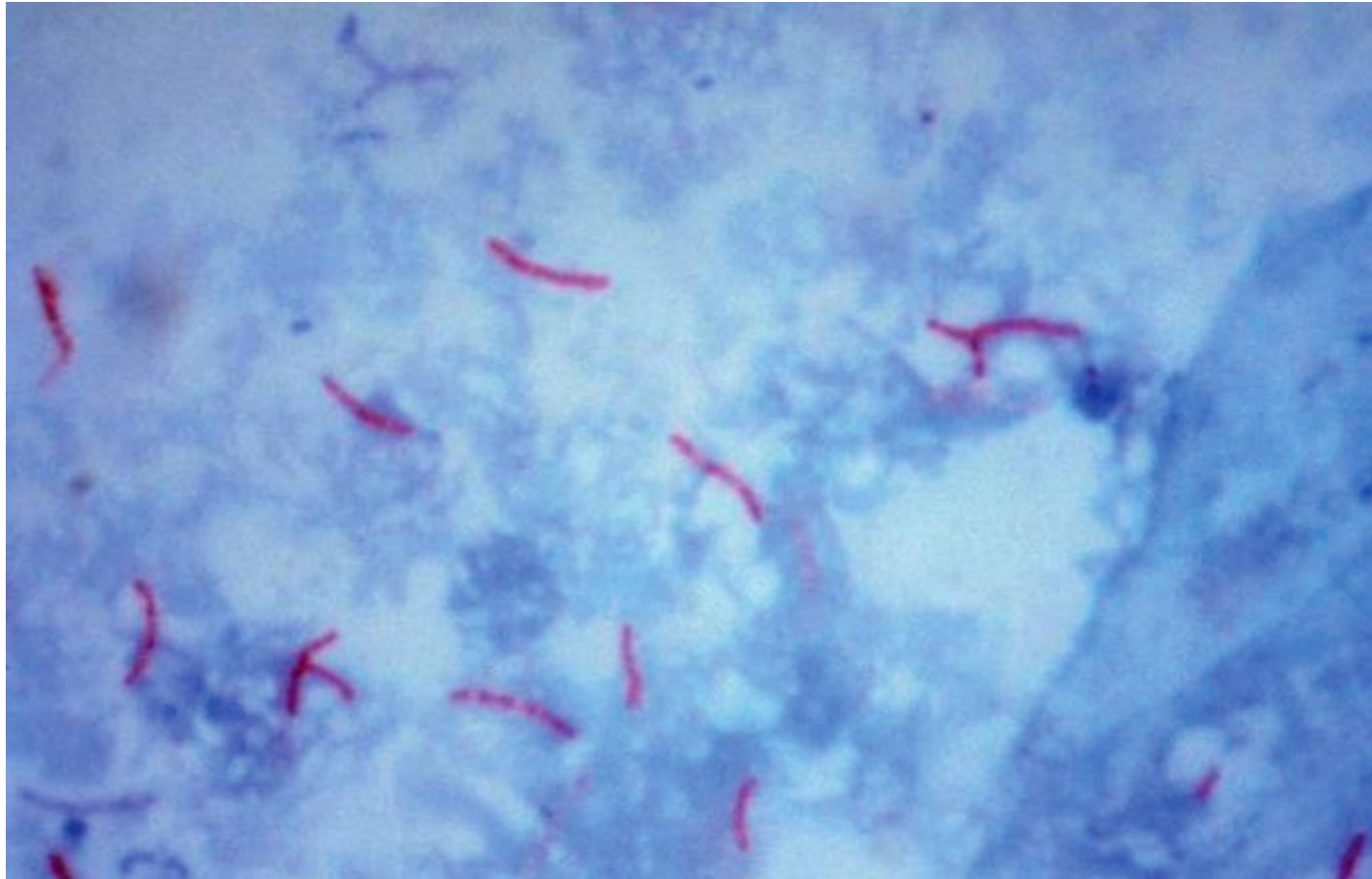
Prevention, and Control

- Leptospirae is excreted in urine both during the active illness and during the asymptomatic carrier state.
- Leptospirae 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 (eg, 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.

Mycobacterium Tuberculosis (Mtb)

- It was not until the 19th century, when Robert Koch utilized a new staining method (ZN stain) and applied it to sputum from patients discovering the causal agent of the disease Tuberculosis (TB); Mtb or Koch bacillus.
- Tuberculosis, consumption (consume patients, weight loss), white plaque (extreme pallor seen among patients)
- The family mycobacterium tuberculosis complex (MTC) can cause Tuberculosis (TB) in humans and other living beings.
- It includes *M. tuberculosis* (Mtb), *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium caprae*, *Mycobacterium pinnipedii*, *Mycobacterium suricattae*, *Mycobacterium mungi*, *Mycobacterium goodii*, *Mycobacterium orygis* and *Mycobacterium canettii*.
- Mtb is a slow growing, obligate aerobe, facultative intracellular bacterium.
- Non-spore forming, non-motile acid fast bacilli.

Mycobacterium Tuberculosis (Mtb) staining



Epidemiology

- Two TB-related conditions exist; latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal. People who have latent TB infection do not feel sick, do not have any symptoms, and cannot spread TB to others
- About one third of the worlds population is infected with TB bacteria (TB latency).
- However, only small proportion of those infected will become sick with TB.
- TB remains a leading cause of infectious diseases morbidity and mortality. In 2015, an estimated 10.4 million new TB cases were seen world wide.
- TB is considered an airborne infectious disease although *M. tuberculosis* complex organisms can be spread through un-pasteurised milk, direct inoculation and other means.

Tuberculosis TB

- The primary site of TB is usually lung, from which it can get disseminated into other parts of the body. The other routes of spread can be contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extrapulmonary organ.
- TB bacteria can attack any part of the body such as the pleura ,L.N. ,pericardium, kidney, spine, brain and abdomen (abdominal Tuberculosis) collectively known as extrapulmonary TB.
- The abdominal TB, which is not so commonly seen as pulmonary TB, can be a source of significant morbidity and mortality and is usually diagnosed late due to its nonspecific clinical presentation.
- The abdominal TB usually occurs in four forms: tuberculous lymphadenopathy, peritoneal tuberculosis, gastrointestinal (GI) tuberculosis and visceral tuberculosis involving the solid organs

Gastrointestinal (GI) tuberculosis pathogenesis

- Abdominal tuberculosis (TB) includes involvement of the gastrointestinal tract, peritoneum, lymph nodes, and/or solid organs .Abdominal TB comprises around 5 percent of all cases of TB
- Tuberculosis of the abdomen may occur via reactivation of latent TB infection or by ingestion of tuberculous mycobacteria (as with ingestion of unpasteurized milk, or sputum or undercooked meat). In the setting of active pulmonary TB or miliary TB, abdominal involvement may develop via hematogenous spread via contiguous spread of TB from adjacent organs (such as retrograde spread from the fallopian tubes) or via spread through lymphatic channels
- The mucosal layer of the GI tract can be infected with the bacilli with formation of epithelioid tubercles in the lymphoid tissue of the submucosa. After 2-4 wk, caseous necrosis of the tubercles leads to ulceration of the overlying mucosa which can later spread into the deeper layers and into the adjacent lymph nodes and into peritoneum. Rarely, these bacilli can enter into the portal circulation or into hepatic artery to involve solid organs like liver, pancreas and spleen

Gastrointestinal TB clinical finding

- The clinical presentation tends to be non-specific, with abdominal pains and general complaints.
- Although any portion of the gastrointestinal tract may be affected, the terminal ileum and the cecum are the sites most commonly involved. Abdominal pain (at times similar to that associated with appendicitis) and swelling, obstruction, hematochezia, and a palpable mass in the abdomen are common findings at presentation. Fever, weight loss, anorexia, and night sweats are also common.

Laboratory diagnostic methods

❖ Smear microscopy

- Three specimens from each patient with suspected TB should be examined microscopically for Acid Fast Bacilli AFB (classically Ziehl-Neelsen) or mycobacteria can be demonstrated by yellow fluorescence after staining with auramin.

❖ Culture

- Both liquid and solid mycobacterial cultures should be performed for every specimen, and recovered isolates should be Caccording to standard criteria (Lowenstein-Jensen or Middlebrook 7H10), Radiometric broth culture (BACTEC radiometric system). mycobacterial growth indicator tube (MGIT).
- Culture for acid fast bacilli is the most specific test for TB and allows direct identification and determination of susceptibility of the causative organism
- ❖ A nucleic acid amplification test (NAAT), Tuberculin skin tests (TSTs), Interferon-gamma release assays (IGRAs) are commonly used as well.

Treatment

- The course of TB treatment depends on whether the individual is in the latent or active stage, and on his or her probability of risk.
- Treatment of TB usually involves a drug cocktail, or a mixture of multiple drugs, with an intensive initial 2-month phase followed by a slower 4- to 6-month continuation phase the main anti-tuberculosis drugs used in the chemotherapy of TB are: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM).
- Isoniazid preventive therapy IPT is the recommended treatment for LTBI but the regimen's main drawback is the duration of therapy

Prevention

- The best way to prevent TB is to diagnose and isolate infectious cases rapidly and to administer appropriate treatment until patients are rendered noninfectious (usually 2–4 weeks after the start of proper treatment) and the disease is cured.
- Additional strategies include BCG vaccination and treatment of persons with LTBI who are at high risk of developing active disease.
- *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG), an attenuated vaccine derived from *M. bovis*, is the only licensed vaccine against tuberculosis (TB)

The End