The Brucellae, Leptospira and Mycobacterium of the GIT

 The Brucellae are Gram-negative, <u>catalase- and oxidase-positive</u>, obligate intracellular organisms, non motile and non spore forming. They are the causative agents of <u>brucellosis</u>.

Brucellosis has three other names, each of which is indicative of a characteristic of the disease:

• Brucellosis is also termed <u>'Malta fever' or 'Mediterranean fever'</u>: this tells you that the disease is highly endemic, and that we are part of the affected region. Interestingly, medical students were taught that any patient with <u>high fever and abnormal gait (limping while walking)</u> has brucellosis until proven otherwise.

• Another term for brucellosis is <u>'undulent fever'</u>: 'Undulent' means (continuously rising and falling) experienced by patients with brucellosis. They could experience high fever in the morning that continues to rise through the afternoon, followed by a drop in temperature in the evening. The cycle then repeats in the following days. They also suffer from excessive sweating (hyperhidrosis) characterized by musty odor (sweat smelling of rot/decay).

There are eight species of Brucellae, but only four cause brucellosis in humans and are of clinical importance:

- o Brucella melitensis
- o Brucella abortus
- o Brucella suis
- o Brucella canis

 Some textbooks describe Brucella as a single species (Brucella melitensis) but with multiple biovars However, we are expected to know that they are four species, each of which has a preferred host • Brucella melitensis produces <u>the most severe</u> and <u>the most</u> <u>common form</u> of brucellosis. It is transmitted via sheep and goat products, like unpasteurized milk, yoghurt or cheese.

• Brucella suis (<u>causing chronic brucellosis</u>) is associated with swine (pigs) and is transmitted to humans by direct contact with swine.

Brucella abortus is associated with cattle and is named as such because it causes abortion in cattle. It does not cause abortion in <u>humans</u>. This is because cattle placentas contain erythritol, a protein necessary for the growth of Brucella abortus, causing placentitis and septic abortion. Human placentas lack this protein. <u>Acute brucellosis</u> caused by this particular species is notorious for its <u>severity</u>.

 Brucella canis is associated with dogs The risk of transmission is particularly high for those dealing with dogs during whelping (giving birth). This species of Brucella causes <u>the mildest form of brucellosis</u>.

However, culturing is the only diagnostic option for this particular species as it cannot be detected by serology.

Therefore, we can sum up the methods of transmission for Brucella as either dealing with the animal directly, or by consuming animal products. It is very difficult to perform studies on Brucella as contracting it significantly deteriorates one's quality of life.

Brucellosis may be acquired 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) ingestion, inhalation, or mucosal (mucous membranes include those of the nose, mouth and conjunctiva) or percutaneous exposure, or even skin abrasions/cuts, sexual transmission, or vertical transmission (from mother to fetus) In addition to all these methods of transmission, Brucella poses an <u>occupational hazard</u> (خطر مرتبط بالمهنة); laboratory technicians, farmers, butchers, and herders all deal with animals directly whether through blood, tissue, or even droplets/splashes that can be inhaled or can come into contact with the conjunctiva or the oral mucosa. This is why laboratory technicians dislike working on samples that are suspected to carry Brucella .

Regardless of how it enters, it crosses tissue barrier and gets ingulfed by resident macrophages or circulating monocytes (recall that they are obligate intracellular bacteria). These circulating monocytes will travel in the bloodstream to the reticuloendothelial system (RES, which includes the lymph nodes, spleen, liver, and bone marrow). This is known as the 'bacteremic trip' of Brucella and is the ideal time for obtaining a blood specimen that is <u>positive upon culturing</u>, especially during acute brucellosis.

- Whereas B abortus requires 5–10% CO2 for growth, the other three species grow in air. <u>All species of Brucella are aerobic except for</u>
 <u>Brucella abortus</u>, which is microaerophilic and capnophilic (requires a high concentration of carbon dioxide for growth)
- They are killed by boiling and pasteurization but are resistant to freezing and drying

Vaccines against Brucella have been developed for animals, but there are none for humans. Sometimes, veterinarians can accidentally prick themselves with a Brucella vaccine that they are trying to give to an animal. This is a form of percutaneous exposure and will lead to the veterinarian becoming infected with Brucella. This is yet another occupational hazard

The only defense of the body against intracellular bacteria is forming <u>granulomas</u>. This is a type of balance that the body demonstrates to try and enclose the area where bacterial multiplication is taking place <u>using a</u> <u>fibrous ring to limit spread</u>. Therefore, granulomas, and particularly <u>caseating granulomas</u>, are seen in patients with brucellosis (recall that caseating granulomas are characterized by epithelioid and giant cell central necrosis and peripheral fibrosis). Granulomas are also seen in TB infections

Upon completing this bacteremic trip, and depending on where the granuloma forms, Brucella tends to settle in certain parenchymatous organs such as the liver, spleen, large lymph nodes, meninges, gallbladder (causing cholecystitis), and bone (hence the typical presentation of limping that brucellosis patients come with, resulting from brucellosis-associated osteoarthritis and osteomyelitis).

- The incubation period ranges from 1–4 weeks, regardless of the method of exposure to Brucella (ingestion, inhalation, mucosal or percutaneous). The onset is insidious, with malaise (general tiredness), fever, weakness, and sweats. Undulent fever is one of the starting symptoms (continuously oscillating between high and low temperatures).
- The fever usually rises in the afternoon; its fall during the night is accompanied by drenching sweat carrying a musty odor.
- There may be gastrointestinal and nervous symptoms. Lymph nodes enlarge, and the spleen becomes palpable (hepatosplenomegaly may occur, in which the liver and spleen become enlarged).

Once the condition becomes chronic, symptoms become more localized and vary according to age:

young patients develop the typical image of abnormal gait (limping) due to the development of osteoarthritis at the hip joint, whereas the elderly tend to develop lower back pain as it is the vertebral bodies that are involved. More symptoms of chronic brucellosis include low-grade fever, generalized fatigue (malaise), and neurobrucellosis (a term for the psychoneurotic symptoms of brucellosis, namely feelings of anger, frustration, depression, and diminished motivation in life). Therefore, chronic brucellosis significantly diminishes the patient's quality of life.

Diagnostic Laboratory Tests :

A. Specimens serum for serologic tests. Blood samples are the first to be taken from patients with acute brucellosis, whereas patients with chronic brucellosis have bone marrow samples taken, as the bacteremic trip of Brucella is over

➤ B. Culture (brain-heart infusion medium, and chocolate agar)

• Important: a negative culture does not mean that there is no infection of Brucella. It could just be that this particular sample taken at a particular time contained no Brucella.

• Moreover, we need to wait for three weeks to declare a culture negative, as growth of Brucella takes around 6-8 days. This long wait of three weeks poses a problem, as faster intervention during the acute bacteremic phase is more effective than interventions done after the patient develops chronic brucellosis. Therefore, we rely on the serology of Brucella for diagnosis, but the definitive diagnosis can only be achieved through isolation (blood culture or bone marrow). In other words, serology is only relied on if a culture cannot prove the presence of Brucella, but a combination of agglutinating and non agglutinating tests must be used for optimal diagnosis. Agglutinating tests alone are not reliable; non-agglutinating tests such as ELISA are more sensitive and more specific.

≻ 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 parallel to each other (together) about 3 weeks after onset of acute disease, peak at 6–8 weeks

• Agglutination test : The SAT (standard agglutination test) uses the smooth Brucella antigen, where IgG agglutinin titers above 1:80 indicate

active infection. Individuals injected with cholera vaccine may develop agglutination titers to brucellae.

 Non agglutinating test (ELISA) assays which use cytoplasmic proteins as antigens. These assays tend to be more sensitive and specific than the agglutination test

Bear in mind that false positives and false negatives may occur. Causes of false positive results include:

- 1) If the patient has taken one of the three cholera vaccines
- 2) Tularemia
- 3) Prozone phenomenon
- 4) Hypergammaglobulinemia, where human globulins mask the binding between the antigens and antibodies, necessitating the use of anti-human globulins

Treatment & Immunity :

• For best results, treatment must be prolonged because of intracellular infections

doxycycline; (an antibiotic given for acne. Brucellosis patients are given two pills a day) and rifampin (1 g) for 6 weeks (45 days) is recommended.

 As is the case with TB, patients feel much better shortly into treatment and are tempted to stop taking their medications. If they do stop, they may develop chronic brucellosis (unless already diagnosed as chronic!!)

Prevention, and Control :

- Eradication of brucellosis in cattle can be attempted by slaughter (necessary to prevent transmission but the owners are compensated)
- •Active immunization (recall that a Brucella vaccine only exists for animals)

• Control rests on limitation of spread and possible eradication of animal infection, pasteurization of milk and milk products

Leptospira

• Leptospira is caused leptospirosis disease, as with brucellosis leptospirosis has other names including <u>'swamp fever'</u> and <u>'mud fever'</u>. Swamps and mud share a common component: contaminated water. Although mentions of contaminated water typically suggest feco-oral transmission but Leptospira is transmitted through the urine so infected animals they shed it in their urine as well as infected humans usually from the second week post infection even they are asymptomatic.

. This poses an occupational hazard for workers dealing with sewage or the sanitation system, who may contract Leptospira through their conjunctiva, leading to suffusion (extreme eye redness and purulent discharge). Spread may also occur indirectly if soil on which vegetables and fruit are grown becomes contaminated with this urine and the crops are subsequently ingested, or by contaminated pools, or by kayaking or rafting in contaminated water.

Feture:

Leptospirae are tightly coiled, thin ,one end is often bent, forming a hook (question mark appearance). They are motile (possess periplasmic flagella).

Unlike the intracellular Brucellae, Leptospirae are extracellular !!

Leptospirosis is considered the most common zoonotic infection worldwide. Brucellosis is also considered zoonotic. The most important reservoir for Leptospira is rodents, which contaminate soil and the sewer system with their urine.

Epidemiology:

Not all infected with Leptospira will develop leptospirosis disease ,the vast majority of infections with Leptospira cause no (asymptomatic) or only mild disease in humans. A small percentage of infections (~1%) lead to severe, potentially fatal complications .(5-10%) at best develop severe leptospirosis, known as Weil's syndrome. This is characterized by a fatal triad of nephritis, hepatitis, and hemorrhage (especially pulmonary hemorrhage).

• Note that, although only about 10% of cases are severe, almost 1 million severe cases are diagnosed each year. Of this 10%, only a small fraction 1% develop the fatal Weil's disease, especially pulmonary hemorrhage.

Pathogenesis:

Leptospirosis is a biphasic disease, consisting of a leptospiremic phase (in which Leptospira circulates in the blood) and an immune phase (in which Leptospira becomes confined to parenchymatous organs, most commonly the liver and the kidneys, and potentially the endocardia causing endocarditis). This is similar to the acute and chronic phases of brucellosis.

This is the triad of Weil's syndrome; nitrogen retention is a sign of renal failure and represents nephritis, while jaundice represents hepatitis.

Between the two phases exists a window period, in which the patient briefly feels better and all the signs of symptoms of the leptospiremic phase (fever, flu-like illness, possible lymph node enlargement, abdominal pain, nausea, vomiting, and other nonspecific signs and symptoms) die down before the second phase (immune phase) begins. The manifestations of the immune phase depend on the organ involved. Leptospira is not found in the CSF, but a high count of WBCs is, leading to signs of neck stiffness and headache. Diagnostic Laboratory Tests:

- A. Specimens Specimens consist of *blood (to see Leptospira),*CSF (to check for leukocytosis and to rule out other causes of meningitis), or *urine and tissues for microscopic examination and culture.
- B. Microscopic Examination Dark-field examination (to see the spirochete if no electron microscope is available)
- C. Culture (Ellinghausen-McCullough- Johnson- Harris EMJH agar is specific for Leptospira)

• Growth is slow, and cultures should be kept for at least 8 weeks before declaring it negative. Compare this to the 3 weeks needed for a culture to be declared negative for Brucella. With patients with Weil's disease, we cannot wait this long. Therefore, the gold standard diagnostic test for leptospirosis is the microscopic agglutination test (MAT). However, the definitive diagnosis is provided through isolation/culturing. The gold standard diagnostic test simply overcomes the time barrier posed by the definitive diagnostic test.

➤ D. Serology The diagnosis of leptospirosis in most cases is confirmed serologically with microscopic agglutination test (MAT)

Treatment & Immunity :

- Treatment of mild leptospirosis should be with oral doxycycline, ampicillin, or amoxicillin. It can even resolve on its own.
- Severe leptospirosis (those with suspected Weil's syndrome) should be treated with IV penicillin
- Serovar-specific immunity follows infection, but reinfection with different serovars may occur.

Prevention, and Control :

• 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, especially those who face an occupational hazard.

• Vaccines for agricultural and companion animals are generally available, and their use should be encouraged. However, there is no vaccine for humans against leptospirosis.