MICROBIOLOGY

بسم الله الرحمن الرحيم

FINAL | Lecture #2

Brucellae & Leptospira

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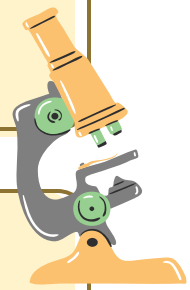

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

Quiz on the previous lecture

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The Brucellae, Leptospira and Mycobacterium of the GIT

By : Nader Alaridah MD, PhD

Think of this scenario as you study the lecture...

A 55-year-old farmer from Irbid, Jordan, presents to the clinic with a two-week history of intermittent fever, back pain, and profuse sweating, particularly at night. He reports consuming unpasteurized goat milk regularly and has been in close contact with his goats. He denies any recent travel or contact with sick individuals. His family members report that he has been feeling angrier and more paranoid than usual. What microorganism could the patient be infected with?

Introduction to the *Brucellae*

- The Brucellae are Gram-negative, obligate intracellular organisms. They are the causative agents of brucellosis. Brucellosis has three other names, each of which is indicative of a characteristic of the disease:
- Brucellosis is also termed 'Malta fever' or 'Mediterranean fever': 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 high fever and abnormal gait (limping while walking) has brucellosis until proven otherwise**.
- Another term for brucellosis is 'undulent fever': 'Undulent' means oscillating and refers to the oscillating fever (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). You wouldn't wish Brucellosis upon your worst enemy!
- There are eight species of *Brucellae*, but only four cause brucellosis in humans and are of clinical importance:
  - *Brucella melitensis*
  - *Brucella abortus*
  - *Brucella suis*
  - *Brucella canis*
- Some textbooks describe Brucella as a single species (Brucella melitensis) but with mutliple biovars (biovars are a group of microorganisms, usually bacteria, that have identical genetic but different biochemical or physiological characters). However, we are expected to know that they are four species, each of which has a preferred host (see next page).

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.
  - Brucella melitensis produces the most severe yet unfortunately the most common form of brucellosis. It is transmitted via sheep and goat products, like unpasteurized milk, yoghurt or cheese.
  - Brucella suis is associated with swine (pigs) and is transmitted to humans by direct contact with swine. It is known for causing chronic brucellosis.
  - Brucella abortus is associated with cattle (الماشية) and is named as such because it causes abortion in cattle. It does not, however, cause abortion in humans. 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. Acute brucellosis caused by this particular species is notorious for its severity.

THE BRUCELLAE

Exam buzzword:
Always associate unpasteurized milk, yoghurt or cheese with brucellosis.

- Brucella canis is associated with dogs (this comes from the scientific name for dogs, *Canis familiaris*). The risk of transmission is particularly high for those dealing with dogs during whelping (giving birth). This species of Brucella causes the mildest form of brucellosis. 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.

- 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. They are obligate intracellular bacteria.
- Whereas B abortus requires 5–10% CO2 for growth, the other three species grow in air. All species of Brucella are aerobic except for *Brucella abortus*, which is microaerophilic and capnophilic (requires a high concentration of carbon dioxide for growth).
- Catalase and oxidase are produced by the four species that infect humans. catalase- and oxidase-positive.
- 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. In addition to all these methods of transmission, *Brucella* poses an occupational hazard; 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*.

Epidemiology

- Brucellosis may be acquired by 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). Regardless of how it enters, it crosses tissue 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 positive upon culturing, especially during acute brucellosis.
- 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. 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.

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. The only defense of the body against intracellular bacteria is forming granulomas. This is a type of balance that the body demonstrates to try and enclose the area where bacterial multiplication is taking place using a fibrous ring to limit spread. Therefore, granulomas, and particularly caseating granulomas, 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, but the TB portion has been excluded from the exam.

Pathogenesis

- 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. 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 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, regardless of the method of exposure to *Brucella* (ingestion, inhalation, mucosal or percutaneous). The onset is insidious, with malaise (general tiredness), fever, weakness, aches, 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). Hepatitis may be accompanied by jaundice.

Clinical Findings

- 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. 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.
- 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. 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
  - 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

Diagnostic Laboratory Tests

- **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.

Diagnostic Laboratory Tests

- 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, and remain high during chronic disease.
    - 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.
    - 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 n the setting of chronic disease.

Diagnostic Laboratory Tests

- 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, also known as rabbit fever, is an infectious disease caused by the bacterium *Francisella tularensis*.

Causes of false negative results include:

1) Prozone phenomenon, an immunologic phenomenon whereby the effectiveness of antibodies to form immune complexes can be impaired when concentrations of an antibody or an antigen are very high.
2) Hypergammaglobulinemia, where human globulins mask the binding between the antigens and antibodies, necessitating the use of anti-human globulins (particularly anti-IgG).

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; an antibiotic given for acne. Brucellosis patients are given two pills a day) and either streptomycin for 2–3 weeks or rifampin (1 g) for 6 weeks (45 days) is recommended.
- Note that intracellular infections always require prolonged treatment.
- 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 test and slaughter (necessary to prevent transmission but the owners are compensated), active immunization of heifers with avirulent live strain 19 (recall that a *Brucella* vaccine only exists for animals), 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 hazardswherever possible.

Leptospira

A very tightly coiled spirochete

- 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).
- As with brucellosis, leptospirosis has other names including 'swamp fever' and 'mud fever'. Swamps and mud share a common component: contaminated water. Although mentions of contaminated water typically suggest feco-oral transmission, *Leptospira* is transmitted through the urine of infected animals and humans instead.

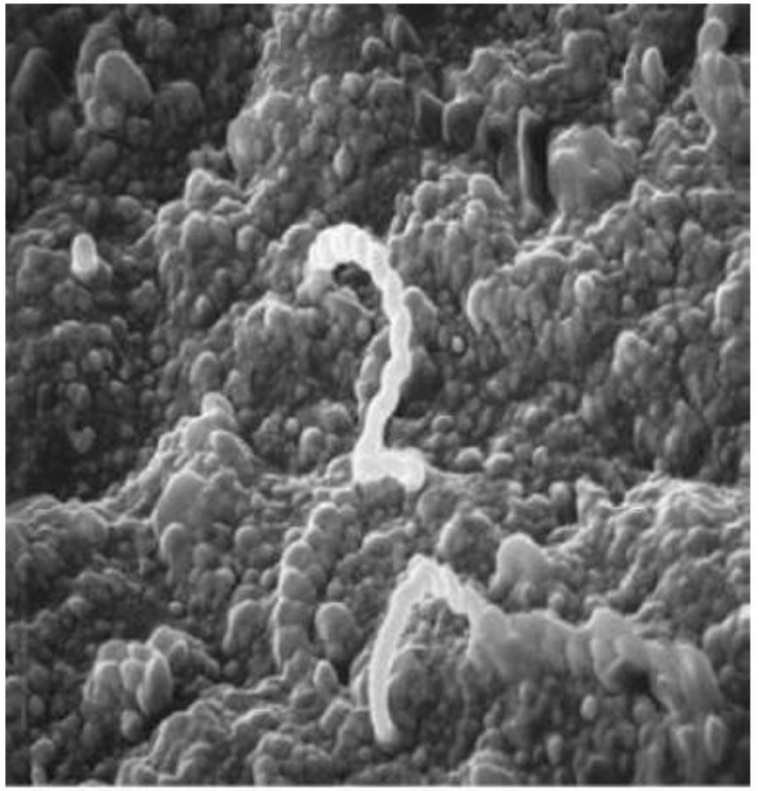
Leptospira

- 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. 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 WITHOUT purulent discharge). Spread may also occur indirectly if soil on which vegetables and fruit are grown becomes contaminated and the crops are subsequently ingested, or by contaminated pools, or by kayaking or rafting in contaminated water.
- Human urine also may contain spirochetes in the second and third weeks of disease even if the individual is asymptomatic.

Leptospira interrogans

Unlike the intracellular *Brucellae*, *Leptospirae* are **extracellular**!!

- 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 (question mark appearance). They are motile (possess periplasmic flagella).
- 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, particularlyat alkaline pH.



Epidemiology

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.

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

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. 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 non-specific 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.
- 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 (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) 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 – EMJH agar is specific for *Leptospira*) in 10 mL test tubes with 0.1% agar and 5-fluorouracil. More regarding culture on next page.

Diagnostic Laboratory Tests

- 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) and ELISA.

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 as soon as the diagnosis is consider.
- 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, 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.

Past Paper Questions

Which of the following is wrong regarding leptospirosis?

a) Varying from asymptomatic to fatal disease
b) Feco-oral transmission
c) Caused by Leptospira interrogans
d) Zoonosis
e) Hepatitis is frequent

Answer: B

Human transmission of *Brucella* can occur by any of the following EXCEPT:

a) Ingestion of infected milk
b) Ingestion of contaminated meat
c) Person to person
d) Inhalation
e) Direct contact with animal tissues

Answer: C

Sketchy for the *Brucellae*

Bruce Farms - Brucella

1. Bruce farms - red to remember it is Gram neg
2. Farm animal is the reservoir - cows and pigs, goats, veterinarian, slaughterhouse worker, or
3. rancher.
4. Milk Bucket on the ground - Indirect contact with milk or cheese products that unpasteurized
5. ~~Open Cage on Barn house - Facultative intracellular can live inside or outside of host cells~~
6. Symptoms - fever, chills, and anorexia initially.
7. Undulating hills - Undulant fever
8. Markings on the cow - Can travel through multiple endothelial organs leading to enlargement of
9. spleen, liver and lymph nodes.
10. Fish Bones - Osteomyelitis - chronic infection
11. Wheel -Treatment - tetracycline, doxycycline
12. Rifle - Along with rifampin for primary treatment
13. blocks oxidative bursts
14. Cage in the background – Infect macrophages
15. Large amounts of catalase and superoxide dismutase to protect from respiratory burst
16. Urease and H2S positive
17. Require cO2 to grow

Sketchy for *Leptospira*

Leptospirosis: The Surfers Oasis

1. Question mark on the board - Spirochetes may be question marked shaped
2. Surfing in the water - Water sports,
3. Yellow tide - water contaminated with animal urine'
4. Surfer rubbing his eyes, rose colored sunglasses and dripping wet - Fever and conjunctival
5. suffusion redness around the eyes without the pus
6. Hawaii - Tropical regions
7. Water
8. Whale – Weil's disease
9. Inner tubes that look like RBC's – travels in blood stream
10. Rubber dingy shaped like a kidney - Renal dysfunction
11. Yellow suit - jaundice from live damage

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	1) The Quiz Form 2) Slide 32 3) Slide 33 4) Slide 18	1) - 2) Point #5: Open Cage on Barn house - Facultative intracellular can live inside or outside of host cells 3) Point #5: Suffusion redness around the eyes without the pus 4) The prozone phenomenon and hypergammaglobulinemia are reasons for false **positive** results	1) One of the answers in the form was corrected (the form now gives the correct answer by default) 2) Crossed out from the *Brucellae* Sketchy was crossed out as it contradicts with what the Professor says 3) Crossed out from the *Leptospira* Sketchy was crossed out as it contradicts with what the Professor says 4) The prozone phenomenon and hypergammaglobulinemia are reasons for false **negative** results
V1 → V2	1) Slides 22 & 33	1) Point #5 was crossed out of the *Leptospira* Sketchy as it contradicts with what the Professor says	1) After contacting the Professor, it seemed that point #5 is in fact scientifically correct and was hence reincluded. It was also fixed on slide 22 (extreme eye redness **and** purulent discharge -> **WITHOUT** purulent discharge)