Summary

- Leptospirosis: bacterial zoonosis
- Transmission via contact with contaminated freshwater
- Fever, muscle pain, cough, red eyes
- Hepatomegaly, icterus, hemorrhagic tendency, meningitis, nephritis
- Difficult clinical diagnosis: water contact, leukocytosis, urine analysis, lumbar puncture
- Serology and direct detection of bacteria are difficult to carry out
- Treatment tetracyclines, penicillin

General

Leptospirosis is the most widespread zoonosis worldwide, caused by the spirochetes of the genus *Leptospira*. An estimated one million people are infected annually, with 60.000 deaths. It is most prevalent in tropical regions, but there are occasional cases in Belgium and the Netherlands. Leptospires are the only pathogenic spirochaetes that are **free-living in the environment**. In comparison, *Treponema pallidum* is only found in humans, and *Borrelia* spirochaetes are only found in arthropods and mammals.

The severe form of leptospirosis was described in 1886 by the German Adolf Weil, Professor of Medicine at the University of Heidelberg. It is therefore still called **Weil's disease**. In 1907 Stimson discovered the organism in kidney tissue from a patient who died during a yellow fever epidemic (see Clinical aspects).

Clinically it is indeed **tough** to differentiate between yellow fever and leptospirosis. In regions where scrub typhus and hantavirosis are endemic, differentiation between *Orientia tsutsugamushi*, hantavirus infection and leptospirosis on clinical criteria alone is impossible.

Taxonomy

The bacteria are very delicate and spiral-shaped. They have a typical terminal hook (Gr. leptos = delicate, slender, speira = spiral, interrogans = question mark). The bacteria are so thin that they cannot be detected with normal light microscopy. They can be seen using phase contrast or dark-field microscopy (urine) and using silver staining of tissue sections.

Leptospires have a characteristic **double membrane architecture with features of both Gram-positive and Gram-negative bacteria**. Traditionally, the genus *Leptospira* contained two species: *Leptospira interrogans* sensu lato, which was pathogenic and *L. biflexa* sensu lato which was non-pathogenic for man. However, the taxonomy of *Leptospiro* has undergone significant changes due to large-scale whole-genome sequencing. There are currently 64 species, split into two clades (pathogenic 'P' and saprophytic 'S') and four subclades. 17 Pathogenic species are classified in subclade P1 (*L. mayottensis, L. alexanderi, L. kirschneri, L. kmetyi, L. alstonii, L. adleri, L. barantonii, L. ellisii, L. dzianensis, L. gomenensis, L. putramalaysiae, L. tipperaryensis, L. borgpetersenii, L. interrogans, L. noguchii, L. santarosai, L. weilii*). Subclade P2 comprises 20 species of intermediate or unclear pathogenicity (*L. broomii, L. licerasiae, L. fainei, L. venezuelensis, L. wolffII, L. haakeii, L. hartskeerlii, L. saintgironsiae, L. neocaledonica, L. perolatii, L. zoumogneensis, L. fletcheri, L. fluminis, L. johnsonii, L. koniamboensis, L. langatensis, L. sarikeiensis, L. selangorensis, L. semungkisensis, L. andrefontaineae, L. inadai*). The previously categorized saprophytes are subdivided in subclades S1 (*L. terpstrae, L. vanthielii, L. yanagawae, L. brenneri, L. harrisiae, L. levettii, L. kemamanensis, L. bandrabouensis, L. bourretii, L. bouyouniensis, L. congkakensis, L. ellinghausenii, L. jelokensis, L. kanakyensis, L. montravelensis, L. mtsangambouensis, L. noumeaensis, L. perdikensis, L. biflexa, L. meyeri, L. wolbachii, L. idonii*) and S2 (*L. ilyithenensis, L. kobayashii, L. ognonii, L. ryugenii*), with 22 and 5 species, respectively. In the older classification, 300 serovars, which can be differentiated by cross-agglutination absorption testing, are grouped into 32 serogroups.

Transmission

The pathogenic bacteria can **survive in freshwater** but die in seawater. Infected animals retain **bacteria in their kidneys for a long time and eliminate them in the urine**. Transmission follows contact with fresh water contaminated with the urine of infected animals. **Rats** form the main reservoir, but other animals, such as cattle, dogs, cats and pigs may also become infected.

Leptospires are killed by gastric acid and bile salts. They penetrate the body via wounds and the mucosa of the mouth, nose and eyes (conjunctivae). **Water** is the most important transmission route, but direct contact with infected animals may also be significant

(slaughterhouse workers, veterinary surgeons). It is a disease associated with **certain occupations**, e.g. workers in paddy fields or on sugar cane plantations, farmers, workers in sewers and canals, gold prospectors (gold dust obtained from water courses). People who bathe or swim in infected surface water are at increased risk of this zoonosis. Now that rafting, kayaking and adventure sports in tropical regions have become popular, there is an increase in leptospirosis in tourists. Ideal conditions for transmission are produced when dirty streets with large rat populations are flooded. Heavy rainfall or flooding in endemic areas can lead to large outbreaks of leptospirosis, especially in areas with poor housing and sanitation. Outbreaks have also been reported in triathlon participants where the swimming was in fresh water.

Clinical aspects

Given the many species of leptospires, **a broad spectrum of diseases is possible**. Symptoms range from mild fever with a 'flu'-like syndrome to atypical pneumonia, myocarditis, aseptic meningitis or the severe Weil's disease with liver and kidney failure, meningitis and hemorrhage.

The disease course has **three phases**: the first **septicaemic**, the second with **leptospiruria** (leptospires in the urine) and the third **convalescence** phase. During the first phase, the leptospires are present in the blood in low numbers (too low to be detected in a blood smear using phase contrast microscopy). Subsequently, the organisms disappear from the blood due to the formation of antibodies. The cellular defense also clears the bacteria from the various tissues. Leptospires persist in the kidney. In the renal tubules, the organisms can multiply and cause renal damage. Bacteria are eliminated with the urine, although the concentration is quite low: $< 10^4$/ml urine. The bacteria may remain in the kidneys for months, even after clinical recovery. Leptospires might also persist in the choroid plexus of the brain.

Most cases of leptospirosis are mild and self-limiting or asymptomatic. Mild forms are often atypical and are generally missed unless they are specifically sought for. The acute phase of leptospirosis usually starts 5 to 14 days after exposure (maximal incubation range 2 to 30 days).

Fever, rigors, myalgias (mainly in the calves and lower back), headache and general malaise usually last two to nine days. Patients can sometimes pinpoint within the hour when the illness began. Next, the fever may subside for a few days and then increase once more (biphasic fever) during the "immune" phase. The absence of this fever pattern does not rule out the disease. Significant muscle pain is almost always present. If it is absent, the diagnosis is improbable.

There is sometimes a sore throat and a dry cough, later possibly hemoptysis. In 10 to 30% of patients, the lower legs have a spotty skin rash. [This was initially described as "Fort Bragg Fever" caused by *L. interrogans autumnalis*]. The eyes are often bloodshot due to dilation of conjunctival blood vessels causing conjunctival erythema. Pus discharge is absent, unlike in purulent conjunctivitis. Subconjunctival hemorrhage can occur on top of the **conjunctival suffusion**. During the immune phase, anterior uveitis presenting as acute onset pain and redness of the eye(s) may occur. Posterior uveitis (chorioretinitis) is less common and presents with decreased vision or floaters. Two-thirds of patients suffer nausea and/or vomiting. Swollen lymph nodes are only present in a minority of patients. The spleen is swollen in 20% of cases.

Marked elevation of CK levels indicates **muscle damage** and occurs only in severe cases. Muscle pain, predominantly in the calves, can lead to local swelling, which can be so severe that patients cannot walk anymore. Pectoral, back and abdominal muscles may also be involved.

Palpation of the calves tends to be painful. The injured muscles heal without scarring. CK levels and muscle symptoms usually diminish in the second week of illness. Rhabdomyolysis seems to be secondary to direct muscle cell invasion with cell necrosis and small intramuscular hemorrhages.

Myocarditis occurs and often leads to congestive heart failure and cardiogenic shock. Electrocardiographic abnormalities are common.

Weil's disease is a syndrome characterized by icteric leptospirosis with fever, jaundice and renal failure. Lung bleeding with ARDS, myocarditis and rhabdomyolysis may accompany this syndrome. **Involvement of the liver** is characterized by hepatomegaly, jaundice and a

hemorrhagic tendency. Scleral icterus and jaundice are accompanied by a marked conjugated bilirubin elevation with normal or slightly elevated aminotransferases. The gall bladder may become inflamed (acute cholecystitis). Liver failure is rare.

Atypical pneumonia with possible blood-tinged sputum can be expected in severe cases. Pulmonary lesions are primarily hemorrhagic rather than inflammatory. Patients are at risk for secondary bacterial pneumonia.

Kidney damage leads to proteinuria, hematuria and uremia. Hypovolaemia and poor renal circulation may further exacerbate renal damage. Hypovolaemia is characterized by oliguria, low blood pressure, diminished skin turgor and flat neck veins. If it is not corrected by giving fluids, tubular necrosis will follow. Temporarily hemodialysis is needed in severe renal failure. Sterile pyuria, proteinuria, granular casts, myoglobinuria and enlarged kidneys occur in some patients. The haem part of myoglobin separates form the globin moiety in an acid environment (pH < 5,4). Renal tubular obstruction due to the precipitation of myoglobin is dangerous. Myoglobin is less toxic if there is no dehydration or acidosis. Therefore alkalinization of the urine is essential.

Meningism may occur early but is more frequent in the immune phase. Neck stiffness is present in half the patients with **aseptic meningitis**. The CSF typically has a neutrophilic or lymphocytic pleocytosis with mild proteinorachia. CSF pleocytosis may last for up to three months. Meningitis is attributed to the immune response rather than a true CNS infection. However, recent studies could *Leptospira* in the CSF by polymerase chain reaction.

In severe leptospirosis, the total period of illness is approximately three weeks to one month. The mortality is between 5 and 30 %; severe icterus has a poor prognosis. If the patient survives, there is usually no residual damage. A long convalescent period is typical.

Differential diagnosis:

This is **very broad because of the variable symptoms**. It includes Hantavirus infection, influenza, gastro-enteritis, meningitis, malaria, hepatitis, cholangitis, rickettsiosis (e.g. scrub typhus), borreliosis, typhoid fever, Reye’s syndrome, arboviroses such as yellow fever, Rift valley Fever, Crimean-Congo hemorrhagic fever and West Nile fever as well as arenaviroses.

In the case of hemorrhagic tendency, Gram-negative bloodstream infections and various viral hemorrhagic fevers should be considered.

Diagnosis

Confirming leptospirosis is quite **difficult**, and the disease is often missed. The disease should be clinically suspected in patients exposed to endemic or outbreak settings who present with systemic febrile illness without an alternative explanation. **Exposure** to potentially contaminated water (occupation, accident, swimming, recent travel to flooded areas etc.) and rat exposure should be enquired. Aseptic meningitis, uveitis, jaundice, acute febrile kidney injury, pulmonary hemorrhage and conjunctival suffusion should raise the suspicion for leptospirosis.

Microscopy of Leptospira sp., bacteria that cause leptospirosis. Photo Cochabamba, Bolivia

There is proteinuria, pyuria and microscopic haematuria. The cerebrospinal fluid initially contains neutrophils. Later, lymphocytes predominate, together with elevated protein and normal glucose.

In general, there is significant leukocytosis, but this is not constant. Thrombocytopenia is common. Early in the disease, leptospires can rarely be found in the blood, urine or cerebrospinal fluid (the tests are not very sensitive). Subsequently, the bacteria are only found in the urine.

Since these are very thin organisms (0.1 µm diameter), a dark-field microscope is needed to detect them in a blood smear. Indirect illumination is used in this method instead of direct illumination so that fine structures can be detected which are not visible with the traditional microscope. This method is not very sensitive and has been responsible for many errors (many false positives and false negatives).

Serology can be performed. The traditional serology using micro-agglutination test or MAT requires a well-functioning laboratory, which will not be available in practice in low resources settings. A single positive or negative IgM or IgG cannot confirm or rule out infection, even though a single IgG titer (>1:800 on MAT) strongly supports infection. A second sample 7 to 14 days after the first antibody test should be obtained, and a four-fold increase in IgG titer confirms infection. Antibody tests that do not detect all serovars may produce false negative results. The interpretation of MAT serology results to identify the responsible serovars is rather difficult because the highest titer does not necessarily correlate with the actual serovar responsible for the infection.

Culture of the bacteria is the gold standard but is **not practical in most settings**. The culture of leptospires is complex and requires non-standard equipment. Special media such as Fletcher’s, Ellinghausen’s, or polysorbate 80 media are required for isolation. Blood and CSF specimens are positive during the first ten days of the illness. Urine cultures become positive during the second week of illness and remain so for up to 30 days after the resolution of symptoms.

In high-resource settings, **PCR** is used on blood samples, urine, CSF and tissue biopsies. Whereas PCR detects leptospires during the first week of symptoms, urine samples are

particularly valuable beyond the first week of illness. The sensitivity of PCR ranges from 40 to 60 percent in blood samples; the specificity exceeds 95 percent.

Antigen detection using a monoclonal antibody-based direct ELISA (anti-*LipL32* antibodies) on blood has shown promising results in Sri Lanka but needs validation in larger international studies.

Treatment

Most patients with leptospirosis will recover without antibiotics. Although robust evidence is lacking, **early treatment initiation** might prevent evolution to severe disease. Antibiotics such as tetracyclines within the first 4 days are assumed to shorten the illness. Sometimes leptospires persist in urine, despite the correct treatment. Oral doxycycline 200 mg per day for 1 week is the preferred regimen for mild infections. If there is vomiting, IV penicillin is used. For severe infections, ceftriaxone can also be used. This allows for once-daily dosing, which is more practical than the multiple dosing schemes using penicillin. Azithromycin and ampicillin are also active against leptospires. Chloramphenicol is not. Most patients with critical illness will have been placed on an empirical antibiotic treatment. However, since the pathophysiology suggest an exaggerated immune reaction, the beneficial effect in severe disease remains controversial.

The immune-triggered second phase suggests a role for corticoids in Weil’s disease. Some studies suggest a possible benefit, but more studies are needed.

Symptomatic and supportive therapy is vital. If there is myoglobinemia, alkalinization of the urine is essential to limit renal damage. In severe disease, hemodialysis, mechanical ventilation and blood products can be life-saving.

Prevention

Since **rats** form the main reservoir and contaminate surface water and drains, their control is important for prevention. Nevertheless, it should not be forgotten that the animal reservoir is much broader (e.g. dogs etc.) and **cannot be eradicated completely**. Avoiding sources of infections, such as water contaminated with animal urine, is advised. Wearing boots when

working in stagnant water is advisable. **Chemoprophylaxis** of 200 mg doxycycline per week may be taken as a preventative in high-risk situations like flooding in an endemic region. After infection, there is protection against the infecting serovar but no cross-immunity. Human vaccines have been developed, but they are serovar-specific, and none of them is widely available. Animal vaccination can provide variable levels of protection for animals and humans.