21 *Leptospira* spp.

A Staff Sergeant was repatriated from an exercise in Belize and admitted to hospital in the UK. He was complaining of fever, headache, and **myalgia**. After an initial improvement, he began to deteriorate. He became jaundiced with signs of **pneumonia** and, on examination, had conjunctival inflammation and hepatosplenomegaly. A chest X-ray indicated bi-basal opacities. His blood count showed a **neutrophilia** with a **thrombocytopenia**. Liver function tests showed an elevated conjugated **bilirubin** with mild elevation of transaminases. He was **oliguric** and **uremic**. A diagnosis of Weil's disease (leptospirosis) was made and he was started on benzylpenicillin and renal dialysis.

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

Leptospira spp. (leptospires) are motile, very thin, tightly coiled spirochetes measuring 0.1 µm by 10–20 µm with a characteristic curve at either end (**Figure 21.1**) and they have a typical gram-negative cell-wall structure (see Case 11, *E. coli*). They have two axial flagella located between the peptidoglycan and an outer-membrane layer in the periplasmic space (**Figure 21.2**).

Ellinghausen McCullough Johnson and Harris (EMJH) liquid medium is used to isolate *Leptospira* and consists of bovine serum albumin, Tween 80, glycerol, sodium pyruvate, cyanocobalamin, and various salts (Mg, Fe, Zn, Ca) dissolved in ultrapure water. Growth is slow and may achieve 10^7 /ml of organism. In some cases, excessive growth can lead to lysis due to the lipases produced by the organism. The generation time can range from 6 to 16 hours depending on the isolate. The organism is aerobic but requires CO_2 to stimulate growth. Maximal growth is between pH 7.2 and pH 7.6.

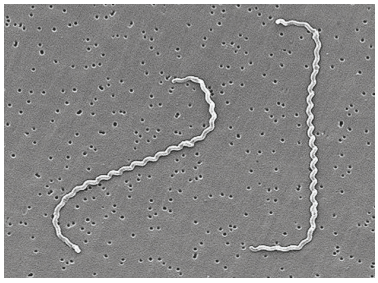

Figure 21.1 Morphology of *Leptospira*. *Reprint permission kindly given by the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #1220. Additional photographic credit is given to Janice Haney Carr who took the image and the CDC NCID and Rob Weyant who provided the image for CDC PHIL website.*

Growth temperature depends on whether it is a pathogen (29°C–37°C) or a saprophyte which can grow at 14°C but not at 37°C .

In addition to the growth temperature indicating its identity as a saprophyte or pathogen, growth in EMJH medium containing 8-azaguanine (or copper) allows the growth of saprophytes but inhibits pathogen growth.

Recently in 2019, the taxonomy of the Genus *Leptospira* has undergone a major review. Previously there were 35 recognized species with many named serovars of one pathogenic species *Leptospira interrogans*. The Genus was divided into three divisions: the Saprophytes (e.g. *L. biflexa*), an Intermediate division (*L. fainei*, *L. inadai*) and the Pathogen division (e.g. *L. interrogans*).

This latest study used whole genome sequencing (WGS) (NextSeq 500 (Illumina), Nextra XT Library preparation and CLC Genomics assembly platform) with additional analysis of Average Nucleotide Identity (ANI) and values of the percentages of conserved proteins (COPD). The study included 90 isolates of *Leptospires* from a variety of locations (e.g. Japan, France, Malaysia, Algeria) and identified 30 new *Leptospira* species giving a total of 64 named species. The taxonomy generated by this study identified two major clades and four subclades P1, P2, S1, S2 (see **Table 21.1**). A study of core gene sets identified genes and domains linked with each subclade. The two major clades are identified as saprophytes (S) and pathogens (P). Subclades P1 (Pathogens in humans and animals), P2 (those species previously classified as Intermediate), S1 (saprophytes) S2 a new subclade including *L. idonii*, *L. kobayashii*, *L. ilyithenesis*, and *L. ognonensis*. Species within this group grew at 14°C but not at 37°C and grew well in the presence of 8-azoguanine, both phenotypic characteristics of saprophytes.

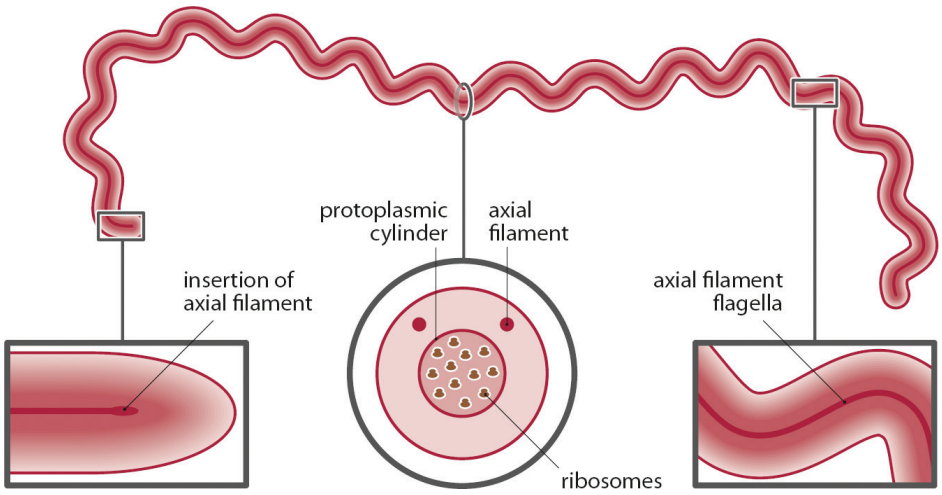

Figure 21.2 The axial internal flagella of *Leptospira*. *Adapted with permission from Dr Samuel Baron and the University of Texas Medical Branch at Galveston, Department of Microbiology and Immunology.*

Table 21.1 Some representative species of *Leptospira*

P1
L. interrogans
L. noguchi
L. alexanderi
L. alstonii
P2
L. licerasiiae
L. hartskeetlii
L. langatensis
L. broomii
S1
L. biflexa
L. levettii
L. perdikensis
L. ellinghauseni
S2
L. idonii
L. ryugenii
L. ilyithenesis
L. kobayashii

ENTRY INTO THE BODY

Infection is acquired by contact with water or soil contaminated by one of the pathogenic *Leptospira* serovars. Entry is via skin lesions, or lesions in the mucosae of the respiratory and digestive tracts or conjunctivae. The organism may also be acquired from contaminated aerosols entering the respiratory tract.

A number of animal species act as a reservoir of infection, the common ones being rodents, but dogs (*L. canicola*) and livestock (*L. pomona*) can also act as reservoirs. Infected animals excrete the bacterium in their urine thus contaminating water sources and soil. Human infection can be acquired directly from contact with the animals or from contact with contaminated water. Globally, *Leptospira* is the commonest **zoonosis**.

SPREAD WITHIN THE BODY

After entry, the organism is spread around the body by the blood, entering all organs and thus giving rise to a wide spectrum of clinical presentations (see later).

EPIDEMIOLOGY

Leptospirosis is a global disease, although it is primarily a disease of tropical and subtropical regions and is relatively uncommon in temperate climates. It is endemic in many countries but outbreaks are also associated with adverse weather. Examples of this include: an outbreak of leptospirosis in Nicaragua following Hurricane Mitch in 1995; an outbreak in Peru and Ecuador following heavy flooding in 1998; a post-cyclone outbreak in Orissa, India in 1999.

The precise number of human cases worldwide is not known but it is estimated that 1.03 million cases and 58 900 deaths occur annually. Incidences range from approximately 0.1–1 per 100 000 per year in temperate climates to 10–100 per 100 000 in the humid tropics; incidence may reach over 100 per 100 000. Reports of cases in the US and Europe are low. In the UK, it varies between 13 and 31 per annum and in continental France is in the order of 300, although in 2005 there were only 212 confirmed cases. In the US, the incidence is between 43 and 93 per annum.

Certain occupations are prone to infection, such as veterinarians, butchers, sewage workers, and farmers. Recreational exposure can occur through adventure holidays such as white-water rafting and other water sports.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

The innate immune system constitutes the first line of host defense, playing a crucial role in early recognition and elimination of leptospires.

INNATE IMMUNITY

Pattern-Recognition Receptors, Neutrophils and Macrophages

Microbes are recognized through their microbial-associated molecular patterns (MAMPs) through a set of different pattern-recognition receptors (PRRs). The MAMPS include many different components such as nucleic acids, flagellin, and lipopolysaccharide (LPS). PRRs also recognize endogenous molecules associated with cellular damage (DAMPs) produced through microbial infection, for example. PRRs are expressed on both immune cells and nonimmune cells (e.g. epithelial cells) and include members of the membrane Toll-like receptor (TLR), the cytosolic NOD-like receptor (NLR) families (Nucleotide-binding oligomerization domain-like receptors (including NOD-1 and NOD-2) and CLR (C-type lectin receptors).

MAMP recognition results in a signaling cascade that leads to activation of transcription factors such as NF-kB and IRF3 that are involved in the production of cytokines, chemokines, and antimicrobial peptides. Pro-inflammatory cytokines produced include interleukins (IL-1β, IL-6, IL-12, interferons (IFNs) and tumor necrosis factors (TNFs), as well as chemokines. Both cytokines and chemokines lead to activation and recruitment of phagocytes, such as neutrophils, macrophages and dendritic cells to the infection site.

Inflammation may not only lead to pathogen destruction but also, if unregulated, can result in a "cytokine storm" observed in patients with sepsis (see pathogenesis).

TLR4 normally senses LPS on gram-negative bacteria but for *Leptospira* LPS, TLR2 appears to be the PRR which can potentially stimulate macrophages to release pro-inflammatory cytokines. Human monocytes are activated, *in vitro*, by *Leptospira* to produce pro-inflammatory cytokines as seen by up-regulation of TNFα and IL1β genes. Intracellular uptake of leptospires through NLR triggers reactive oxygen species (ROS) and reactive nitrogen species (RNS). In addition, infection triggers TLR2-mediated production of IL-8 and NLRP3-dependent production of IL-1β.

TLR2 on human macrophages also recognizes the outer-membrane lipoprotein LipL32, the major lipoprotein of leptospires, It is thought that this occurs through dimers of TLR2 and TLR1.

NOD-1 and NOD-2 (NLR family) are intracellular receptors that recognize peptidoglycan fragments of bacterial peptidoglycans (PGs) called muropeptides and are active against *Leptospira* PGs.

There appears to be some controversy around the importance of neutrophils in the host response to *Leptospira*. Data suggest that direct phagocytosis of leptospires is rather poor although early studies on immunity indicated that serum from infected individuals but not "normal serum" results in phagocytosis. This indicates that antibodies were "opsonizing" the organisms for phagocytosis. It appears that neutrophils are mostly effective against saprophytic leptospires rather than pathogenic leptospires. Neutrophils also release myeloperoxidase and ROS that are antimicrobial.

The antimicrobial peptide cathelicidin released by neutrophils has been shown to have anti-leptospiral activity among different *Leptospira* species (*L. interrogans* serovars and *Leptospira biflexa*). In addition, a particular strain of *L. interrogans* triggers the release of DNA extracellular traps (NETs) and kills leptospires through the process of NETosis – extrusion of the neutrophil DNA with bactericidal proteins which results in trapping and/or killing of the pathogens. It has been suggested that these DNA traps are crucial to preventing early leptospiral dissemination.

Complement

The complement system has an important role in protection against foreign microbes. It is made up of many molecules (around 50) and is activated through three pathways, classical (CP), alternative (AP), and lectin (LP). Both LP and AP pathways are involved in the host innate immunity while the CP pathway is triggered by the IgM or IgG antibodies specifically bound to antigens. The lectin pathway is triggered when lectins, including ficolins or mannose-binding lectin, attach to carbohydrate moieties on the microbial surfaces. Activation of all three pathways leads to promotion of phagocytosis (opsonization), recruitment of inflammatory mediators and inflammation and lysis via the MAC attack complex. Recent data on using inhibitors of these pathways and survival in human serum of non-pathogenic or pathogenic serovars of *Leptospira* have suggested that both AP and LP have an important role in eliminating saprophytic leptospires. In all leptospires investigated, the deposition of the lytic MAC attack complex proteins on saprophytic *Leptospira* strains was more pronounced when compared to pathogenic species. The pathogenic species have many evasion mechanisms targeting complement activation – see below.

γδT cells, that recognize a broad range of antigens without the presence of major histocompatibility complex molecules, have been shown to respond against leptospires. Identification of the protein antigens recognized will be important in understanding their role in the leptospiral immune response.

Adaptive immunity

PRR activation is also important for the development of adaptive immunity and results in the expression of costimulatory molecules at the surface of macrophages and dendritic cells that are important for antigen presentation to naive T cells and the subsequent activation of B cells and the production of antibodies.

Experimental animal models of leptospires have provided most of the evidence for antibodies playing a key role in both protection and clearance of infections. Several studies in leptospirosis patients have shown that there is a strong antibody response to leptospire LPS. Both IgM and IgG antibodies are produced against a variety of leptospire-specific antigens. These include LipL32, LipL41, and leptospiral immunoglobulin-like A (LigA) which are present only in pathogenic leptospires and have been shown to be exposed on their cell surface. The major protective role of IgG antibodies (which is increased in infections with pathogenic leptospires) seems to be opsonization. The role of T cells is unclear except for their help with the production of antibodies.

Host-response evasion mechanisms

Most leptospire infections are asymptomatic and/or resolve fairly quickly, probably as the result of an effective immune response not compromised by extensive evasion mechanisms. Other strong pathogenic leptospires that do have extensive evasion mechanisms produce more severe and sometimes fatal illness – see pathogenesis below.

PRRs: NOD-1 and NOD-2 receptors cannot sense *Leptospira* since its outer-membrane protein, LipL21 lipoprotein, binds to the PG and impairs its degradation into muropeptides, which therefore cannot signal through NOD-1 and NOD-2.

Complement evasion: The complement system provides an early innate defense. Pathogenic leptospires use different sophisticated strategies to subvert or inactivate all three pathways (classical, alternative, and lectin) of the complement cascade.

Pathogenic leptospires evade complement attack by binding Factor H (FH) and C4 binding protein (C4BP) of the alternative and classical pathways onto their surfaces. FH, a plasma protein, inhibits the alternative pathway of complement by preventing binding of Factor B to C3b, accelerating decay of the C3-convertase C4BP and acting as a co-factor for the cleavage of C3b by Factor I. LenA and LenB are leptospiral ligands for human FH. C4BP, a plasma glycoprotein, inhibits the classical pathway of complement by interfering with the assembly and decay of the C3-convertase C4bC2a and acts as a co-factor for Factor I in the proteolytic inactivation of C4b. LcpA is a leptospiral outer-membrane protein which interacts with human C4BP. Thus, complement activation is down-regulated preventing opsonization and the formation of the lytic membrane attack complex on its surface.

Phagocyte function: the outer-membrane proteins of *L. interrogans* serovar Copenhageni – LipL21 and LipL45 have been shown to be myeloperoxidase inhibitors.

Pathogenesis

Leptospirosis starts with an acute phase that is self-resolving in most of the cases and is followed potentially by a chronic phase (especially kidney colonization) depending on the virulence of the strain, severity of the acute phase, and the overall immune defense of the host. As shown above, pathogenic leptospires have many evasion mechanisms to overcome the immune system. Around 10% of leptospirosis cases develop into severe forms.

The organism migrates to the interstitium, renal tubules, and tubular lumen of the kidney causing an interstitial nephritis and tubular necrosis. Liver involvement is seen as centrilobular necrosis with proliferation of Kupffer cells. Leptospires also invade skeletal muscle, causing edema, vacuolization of myofibrils, and focal necrosis. In the lungs, leptospires induce intra-alveolar hemorrhages. A consistent pathologic finding in all organs is a vasculitis. Whether organ damage is due directly to secreted toxins of leptospires or is secondary to the vasculitis induced by cell-wall components of the organism such as collagenase or bystander damage is unclear. A study has shown that the peptidoglycan of leptospires can increase the adhesion of the organism to vascular endothelial cells and is, thus, an important virulence factor. The illness is characterized by a bleeding diathesis although its pathophysiology is uncertain. Infection causes a thrombocytopenia, but it is unclear whether this is due to a direct action of the leptospires on thrombopoiesis or secondary to disseminated intravascular coagulation or a specific anti-platelet antibody.

During infection, the triggering of the inflammatory response through the production of pro-inflammatory cytokines is important for the early elimination of pathogens. However, unregulated cytokine production can result in a cytokine storm that might be followed by a state of immunoparalysis, which can lead to sepsis, associated organ failures, and subsequent death of some patients with severe leptospirosis.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

Leptopirosis was first recognized in sewage workers in 1883. The incubation period is about 10 days but can range from 5 to 30 days. Because the organism spreads to all organs of the body, the clinical presentation may vary. The infection may be asymptomatic and exposure is only recognized serologically. Symptomatic disease may present as a biphasic illness with an initial nonspecific phase where the patient complains of:

- a high temperature with rigors;
- headache;
- myalgia;
- retro-orbital pain and photophobia;
- conjunctivitis;
- nausea, vomiting, diarrhea;
- dry cough;
- pre-tibial rash.

This is the leptospiremic phase of the illness lasting about 7 days. After the temperature falls, more specific system-based

symptoms may develop within a few days clinically presenting as:

- aseptic meningitis (headache, stiff neck, photophobia, lymphocytic CSF);
- hepatitis (jaundice);
- renal failure with jaundice and hemorrhagic features (Weil's disease);
- pulmonary infection (cough, hemoptysis);
- systemic inflammatory syndrome or shock.

The commonest presentation is anicteric, for example aseptic meningitis (80–90%) compared to icteric with renal failure (Weil's Disease 10–20%).

Laboratory abnormalities occur associated with specific syndromes, for example increased serum creatinine, thrombocytopenia, leucocytosis, and hyperbilirubinemia.

On examination, the liver and / or spleen may be enlarged. This phase corresponds to the *immune phase* and may last from 1 to 6 weeks. In some cases, the two phases may not be apparent or the illness appears to start with the immune phase. The severity of the disease may vary from a mild illness to more severe disease (Weil's disease) with complications including cardiac dysrhythmias, liver or renal failure, myelitis, and Guillain-Barré syndrome. Weil's disease carries a high mortality.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

The laboratory diagnosis is made with:

- dark-ground microscopy;
- culture;
- serology;
- genomics.

Dark-Ground Microscopy

Leptospira spp. can be detected by dark-ground microscopy in blood or cerebrospinal fluid (CSF) during the first week of the illness and in the urine about 10 days thereafter. It has a low sensitivity and specificity and requires a concentration of 10^4 *leptospires*/ml for detection.

Culture

Leptospires can also be cultured from the blood, CSF or urine using EMJH medium incubated at 30°C for 6–8 weeks. Because of the low sensitivity and specificity of dark-ground microscopy, and the long timescales of culture, the routine method of diagnosis is serologic. Culture, however, is important in epidemiologic studies with specimens obtained from soil, water, animals, and humans although, for the latter, it is not an important diagnostic test because of the long timescales.

Serology

The microscopic agglutination test (MAT) is difficult to perform and relies on mixing serum from the patient with live leptospires from the different serogroups and looking for agglutination by dark-ground microscopy. This test also has a low sensitivity due to failure to **seroconvert** in a proportion of patients and cross-reactivity with a number of other infections and illnesses including other spirochetal diseases (Lyme disease, relapsing fever, treponemal disease), *Legionella*, HIV, and autoimmune diseases. Although the MAT is serogroup-specific, cross-reactions between serogroups occur and the individual serovars cannot be determined. A result of > 1:100 or a four-fold rise in titer is considered positive.

An IgM ELISA directed against LipL32 is most often used for diagnosis and detects antibodies 7–10 after infection. The majority of IgM (95%) are directed against this antigen and it is common in pathogenic leptospires. It has a high sensitivity and specificity.

Other formats such as latex agglutination, indirect hemagglutination and lateral flow tests (LeptoTek Lateral Flow) are also commercially available and have the advantage that can be used as point-of-care (POC) assays.

Genomics

Various genomic assays are available:

Polymerase chain reaction (PCR) directed against the *sec Y*-gene or the *LipL32* gene have a high sensitivity and specificity. Other PCR modifications are also available, for example chip-based RT-PCR (Real-time PCR), loop-mediated isothermal amplification (LAMP).

Whole Genome Sequencing (WGS) will probably be the standard genomic method of leptospire detection as it has more specificity of the identity of leptospire species. For example, PCR using 16S RNA is useless, as 16 different leptospire species all have the same 16S RNA profile. However, one gene that may prove useful to detect all leptospires is the *ppk* gene (*polyphosphate kinase*) as it evolves rapidly and gives the identical taxonomy to WGS.

DIFFERENTIAL DIAGNOSIS

Leptospirosis is endemic in areas where the following are found:

- Dengue virus (co-infection occurs in about 8% of cases);
- malaria;
- hantavirus;
- scrub typhus.

Patients presenting with the initial febrile illness may be diagnosed with Dengue fever which is more common than leptospirosis. If presenting with signs of meningitis, then leptospirosis may be misdiagnosed as viral meningitis or if with petechiae then meningococcal meningitis. A CSF white cell count would suggest a viral etiology rather than meningococcal, as the cell response in leptospirosis is

lymphocytic. If presenting with jaundice the differential diagnosis includes:

- viral hepatitis;
- malaria;
- schistosomiasis;
- relapsing fever;
- tularemia

and if with jaundice and renal failure then it includes:

- Legionnaires disease;
- hemolytic uremic syndrome.

If the pulmonary syndrome is prominent, it may be confused with hantavirus.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

MANAGEMENT

Antimicrobials

As previously indicated, the majority of cases of leptospirosis are self-limiting and do not require antimicrobial therapy. If the illness is severe enough to cause clinically recognized symptoms and diagnosis is made, antibiotic therapy needs to be started to reduce the duration of illness and shedding of organisms in the urine.

Mild disease: patients are treated with doxycycline or azithromycin, ampicillin or amoxicillin, clarithromycin, fluoroquinolone such as ciprofloxacin or levofloxacin. These antibiotics also have activity against rickettsial disease, which can often be confused with leptospirosis.

Severe disease: for hospitalized patients, IV penicillin, and oral doxycycline for up to 7 days. Ceftriaxone or cefotaxime can also be given.

A Jarisch-Herxheimer reaction may occur following antimicrobial therapy for leptospirosis and is characterized clinically by fever, rigors, and hypotension.

This reaction is due to the release of endotoxin when large numbers of organisms are killed by the antibiotics leading to increase in immune complexes and the release of cytokines, particularly TNF. This reaction also occurs typically with syphilis and Lyme disease. Supportive measures may be required, such as hemodialysis, in severe disease.

There is also some evidence for the benefit of plasmapheresis in severe leptospirosis.

Additional clinical support

There is insufficient data evidence for the routine use of IV steroids but it has been proposed given the vasculitic nature of severe leptospirosis, especially in the setting of pulmonary involvement. Some data have indicated a benefit for the use of steroids as an adjunct to antibiotic therapy in severe disease but further study is needed.

PREVENTION

Prevention includes avoiding potential sources of infection, prophylaxis for individuals at high risk of exposure, and animal vaccination.

The most important control measures for preventing human leptospirosis include avoiding potential sources of infection such as stagnant water and animal farm water runoff, rodent control, and protection of food from animal contamination.

In a study of antimicrobial prophylaxis with doxycycline for individuals at high risk of exposure, fewer cases of clinical leptospirosis were observed in the antibiotic-treated groups of soldiers on jungle exercises versus the placebo group.

Vaccines

The real challenge is to produce a universal vaccine against all of the >300 serovars of *Leptospira*. After many years of research, this has not yet been achieved. Inactivated whole cells (bacterins) are the only vaccines commercially available, primarily for veterinary use. Vaccines that have been produced are mainly serovar dependent and based on lipopolysaccharide antigens. In addition, inactivated vaccines do not promote long-term protection and require annual boosters. Safety has also been reported as an issue. Vaccination of domestic and farm animals against leptospirosis has been shown to give variable levels of protection with some immunized animals becoming infected and excreting leptospires in their urine.

Many approaches are being made to produce effective vaccines and these have been helped by the WGS of leptospires. These include live vaccines, multiepitope vaccines against some OMP, DNA vaccines, and use of NOD-1 and NOD-2 agonists. The development of mRNA vaccines (recently used in SARS-2 vaccines) coding for multiple-surface proteins is also predicted to be an attractive future approach for creating a successful vaccine.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- Two main identified species exist: *L. interrogans* and *L. biflexa*.
- There are two clades – Pathogens and Saprophytes.
- Leptospirosis is a zoonosis, the organism is carried by many animals and excreted in the urine.
- Infection is acquired by contact with contaminated water or soil.
- The organism enters through the conjunctiva, mucosa or skin abrasions.
- Leptospires spread to all body locations by the blood.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- TLR2 senses outer-membrane protein LipL32 and NOD-like receptors sense peptidoglycan fragments of leptospires. Stimulation through these receptors results in production of pro-inflammatory cytokines TNFα, IL1β and IL8 leading to recruitment of neutrophils, macrophages and dendritic cells to the site of infection.
- The antimicrobial peptide cathelicidin released by neutrophils is effective against some leptospires. Neutrophils also release myeloperoxidase and ROS.
- Complement is important as a host mechanism against leptospires, especially against the non-pathogenic serovars (saprophytes).
- A strong antibody response is made against leptospire LPS. Both IgM and IgG antibodies are made against a variety of specific antigens including LipL32, LipL41, and LigA. The major role of IgG seems to be in opsonization.
- Leptospires, especially those that are pathogenic, have many ways of evading the immune system including: inhibition of degradation of PG into muropeptides that are not recognized by NOD-1 and NOD-2 receptors; subversion or inactivation of complement pathways.
- Pathogenesis: around 10% of leptospirosis cases develop into severe forms. Pathogenic leptospires cause vasculitis in many organs. Liver, lungs, heart, meninges, kidneys, and muscles are all affected; Thrombocytopenia can occur.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- The incubation period is about 10 days but can be 5–30 days.
- The clinical presentation is classically biphasic.
- The initial illness is nonspecific with fever and myalgia.
- The second phase of the illness may present as meningitis, pneumonia, jaundice or renal failure.
- Complications include cardiac arrhythmia, Guillain-Barré syndrome and renal failure.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- Leptospires can be detected in the blood, CSF or urine by dark-ground microscopy.
- The organism can be cultured from the blood, CSF or urine using EMJH medium incubated at 30°C for 6–8 weeks.
- Serologically, the disease can be diagnosed by the macroscopic agglutination test (MALT) or the IgM ELISA.
- Various genomic assays exist: PCR, RT-PCR (real-time PCR), LAMP, and WGS.
- The differential diagnosis includes Dengue virus, viral meningitis, viral hepatitis, malaria, hantavirus and Legionnaires disease.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- Most cases of leptospirosis are self-limiting.
- Patients with mild disease are treated with doxycycline or azithromycin.
- Hospitalized patients with severe cases are given IV penicillin, and oral doxycycline and ceftriaxone or cefotaxime is given for up to 7 days.
- A Jarisch-Herxheimer reaction may occur following antimicrobial therapy and is characterized clinically by fever, rigors, and hypotension.
- Prevention is by identifying risks, antimicrobial prophylaxis for individuals at high risk, and animal immunization (although shown to have variable levels of protection).
- Vaccines pose a major challenge because of the many different serovars. There are many new approaches, helped by WGS, and include live vaccines against some OMP, DNA vaccines, and the use of NOD-1 and 2 agonists and mRNA.

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European Centre for Disease Prevention and Control, Leptospirosis: https://www.ecdc.europa.eu/en/leptospirosis

GOV.UK, Leptospirosis, 2013: https://www.gov.uk/guidance/leptospirosis

Medscape, Leptospirosis, 2021: https://emedicine.medscape.com/article/220563-overview

The Leptospirosis Information Center, 2008: http://www.leptospirosis.org/

Students can test their knowledge of this case study by visiting the Instructor and Student Resources: [www.routledge.com/cw/lydyard] where several multiple choice questions can be found.