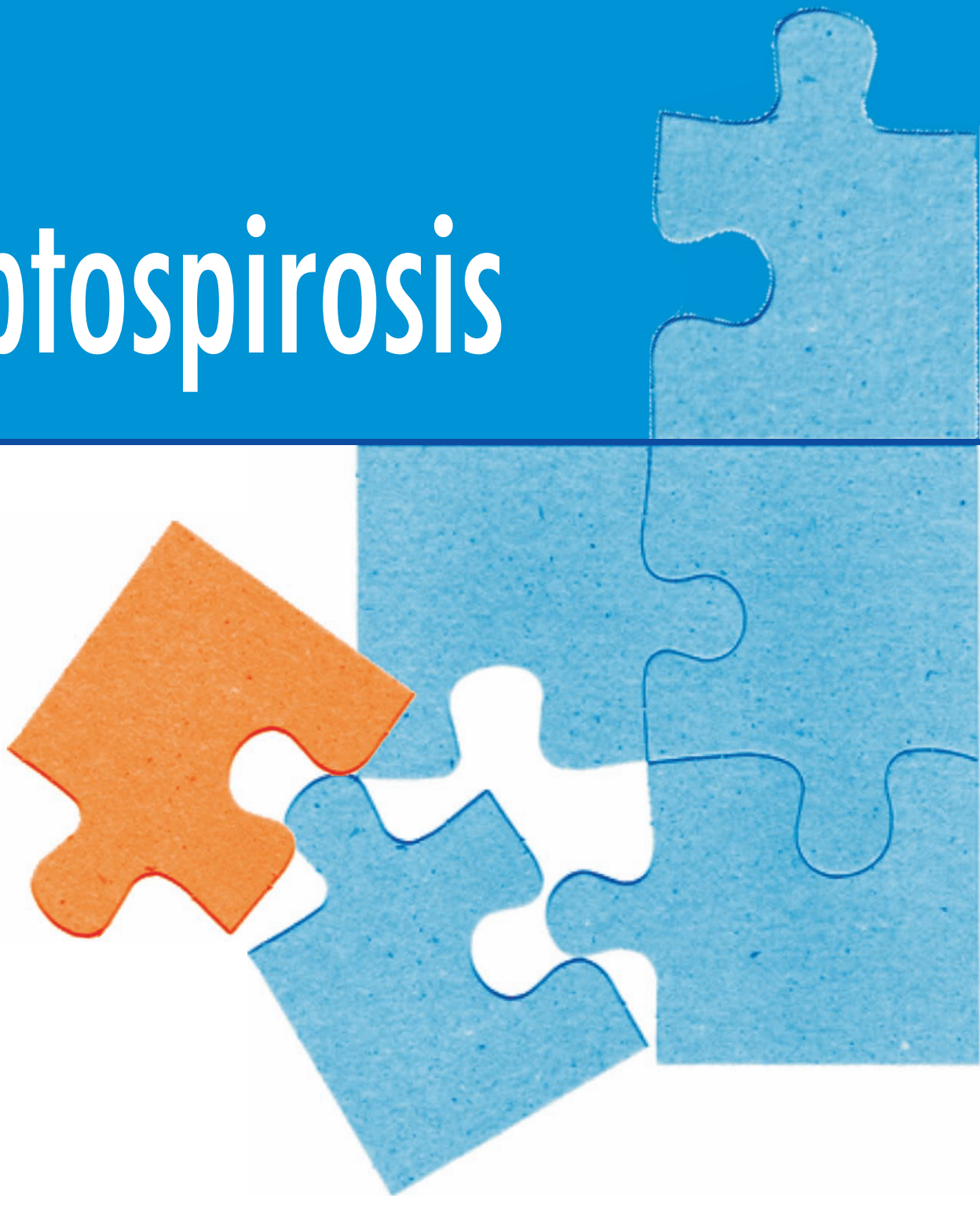
FACT SHEET

# Leptospirosis



World Health Organization
Regional Office for South-East Asia

# Introduction

## What is leptospirosis?

Leptospirosis is an infectious disease caused by pathogenic organisms belonging to the genus *Leptospira*, that are transmitted directly or indirectly from animals to humans. Leptospirosis is a major direct zoonosis. Human-to-human transmission occurs only very rarely.

## What are leptospires?

Leptospires are bacteria which can be either pathogenic (i.e. having the potential to cause disease in animals and humans) or saprophytic (i.e. free living and generally considered not to cause disease). Pathogenic leptospires are maintained in nature in the renal tubules and genital tracts of certain animals.

## Can pathogenic leptospires be distinguished from one another?

Yes. The basic systematic unit is the serovar, defined on the basis of antigenic similarities and differences. Each serovar has a characteristic antigenic make-up. Prior to the development of DNA analysis, classification was done by serological cross agglutination absorption-testing (using serum antibodies to identify similar or different types of bacteria). Today Lipopolysaccharide (LPS) is the major antigen involved in serological classification. Structural heterogeneity in the carbohydrate component of LPS moieties derived from differences in the genes involved in LPS biosynthesis appears to be the basis for a large degree of antigenic variation observed among serovars.

The current practice is that pathogenic strains are assumed to be serovars of L. interrogans, while non-pathogenic are assigned as L. biflexa.

## How many pathogenic serovars exists and how are they characterized?

So far, there are over 250 pathogenic serovars. Serovars having antigenic similarities are formed into serogroups, and all serovars have been divided into 25 serogroups. Different strains with small antigenic differences can sometimes be found within certain serovars.

## Which animals are considered as reservoirs of leptospirosis?

Virtually all mammalian species can harbour leptospires in their kidneys and act as source of infection to human beings and other animals. However, cattle, buffaloes, horses, sheep, goat, pigs, dogs and rodents are common reservoirs of leptospires. Rodents were the first recognized carriers of leptospirosis. They are the only major animal species that can shed leptospires throughout their lifespan without clinical manifestations, i.e. prolonged carrier state. They are incriminated as a primary source of infection to human beings. Although serovars Ichterohaemorrhagiae, Copenhageni, Grippotyphosa and Ballum have been often associated with rodents, other serovars have also been isolated. Pigs and cattle can excrete very large amounts of leptospires in the carrier state (i.e., chronic leptospiral colonization of the renal tubules) and can be an important source of human infection.

## Do all animals infected with leptospires become sick?

No. Animals that are natural hosts to a particular serovar usually show no or comparatively few ill effects after infection with that serovar. However, they may develop illness after infection with another serovar.

Chronic infections in animals may lead to reproductive problems, such as abortion and low fertility in cattle or pigs. Occasionally, calves and piglets may suffer from an icterohaemorrhagic syndrome with potentially fatal outcome. Dogs may suffer from a chronic disease leading to kidney damage, but may also suffer from an acute Weil's-like disease syndrome after infection with certain serovars.

# Epidemiology

## Where does leptospirosis occur?

Leptospirosis occurs worldwide but is most common in tropical and subtropical areas with high rainfall. The disease is found mainly wherever humans come into contact with the urine of infected animals or a urine-polluted environment.

## How often does leptospirosis occur in the South-East Asia Region?

Leptospirosis is reported in a number of countries of the South-East Asia Region from time to time. The magnitude of the leptospirosis problem differs from country to country and depends on awareness and attitude of public heath care decision makers. Most human cases have been reported from India, Indonesia, Thailand and Sri Lanka during the rainy season. Major outbreaks in South-East Asia were reported in the past in Jakarta (2003), Mumbai (2005) and Sri Lanka (2008). Seasonal outbreaks are reported in northern Thailand and in Gujarat, India following heavy rainfall and flooding. A few human cases have been reported from Maldives. According to currently available reports, incidences range from approximately 0.1–10 per 100 000 per year globally. During outbreaks and in high-exposure risk groups, disease incidence may reach over 50 per 100 000.

There are anecdotal reports of human and animal cases in Bangladesh, Myanmar, Nepal and Timor-Leste. However, no information is available about leptospirosis in Bhutan and Democratic People's Republic of Korea.

## Why is human leptospirosis neglected and underreported?

Leptospirosis may present with a wide variety of clinical manifestations. These may range from a mild "flu"-like illness to a serious and sometimes fatal disease. It may also mimic many other diseases, e.g. dengue fever, typhoid, viral hepatitis and other viral haemorrhagic diseases. Icterus (jaundice) is a relatively common symptom in leptospirosis but is also found in many other diseases involving the liver such as various forms of hepatitis. They are, therefore, often not recognized as a presenting feature of leptospirosis. Icterus may be totally absent even in some of the severe cases that present with complications involving other organ systems such as severe pulmonary haemorrhage, which develop early in the course of the disease. Such cases are being reported in increasing frequency from various places in the recent years.. The diagnosis is confirmed by laboratory tests, but these are not always available. For these reasons, leptospirosis is neglected and underreported.

## Is leptospirosis an endemic or an epidemic disease?

Leptospirosis is endemic in many countries, across the world. It often has a seasonal distribution, increasing with increased rainfall or higher temperature. However, the disease can occur throughout the year. Epidemics may be associated with changes in human behaviour, animal or sewage contamination of water, changes in animal reservoir density, or follow natural disasters such as cyclones and floods.

## Have all humans an equal chance of becoming infected with leptospires?

The risk of infection depends on exposure. In fact, some humans have a high risk of exposure because of their occupation, the environment they live in or their life-style and risk groups can be defined on this basis. The main occupational groups at risk include farm and agricultural workers, pet shop workers, veterinarians, sewer workers, abattoir workers, meat handlers, and the military. Other groups at high risk of contracting leptospirosis include survivors of natural disasters (e.g. flooding) and the increasing number of people engaging in recreational water sports. In some countries, practically the whole population is at risk as a result of high exposure to contaminated water in daily activities, e.g. paddy and sugarcane plantation.

# Mode of transmission

## What is the mode of transmission of leptospirosis?

Human leptospiral infections result primarily from direct or indirect exposure to the urine of infected animals.

Moisture is an important factor of the survival of the leptospires in the environment. Other modes of transmission of infection, such as handling infected animal tissues and ingestion of contaminated food and water, are also possible.

## How do leptospires enter the body of humans and animals?

Leptospires can gain entry into humans through cuts and abrasions in the skin, through intact mucous membranes (nose, mouth, eyes) and perhaps through waterlogged skin. They may occasionally enter the human body via the inhalation of droplets of urine or via drinking-water.

## What happens to the pathogenic leptospires after they have penetrated the human body?

After infection, leptospires appear in the blood and invade practically all tissues and organs. They are subsequently cleared from the body by the host's immune response to the infection. However, they may settle in the convoluted tubules of the kidneys and be shed in the urine for a period of a few weeks to several months and occasionally even longer. They are then cleared from the kidneys and other organs but may persist in the eyes for much longer.

## Can leptospires be transmitted from human to human?

Yes, but rarely. They can be transmitted from human to human by sexual intercourse, transplacentally from the mother to the fetus and via breast milk to a child. Urine from a patient suffering from leptospirosis should be considered infectious. As leptospires can be cultured from blood, this should be viewed as infectious for some time before the onset of symptoms and during the first 7–10 days of illness.

# Clinical Manifestations

## What are the clinical manifestations of leptospirosis?

The clinical manifestations are highly variable. In general, the disease presents in four broad clinical categories:

(i) a mild, influenza-like illness;

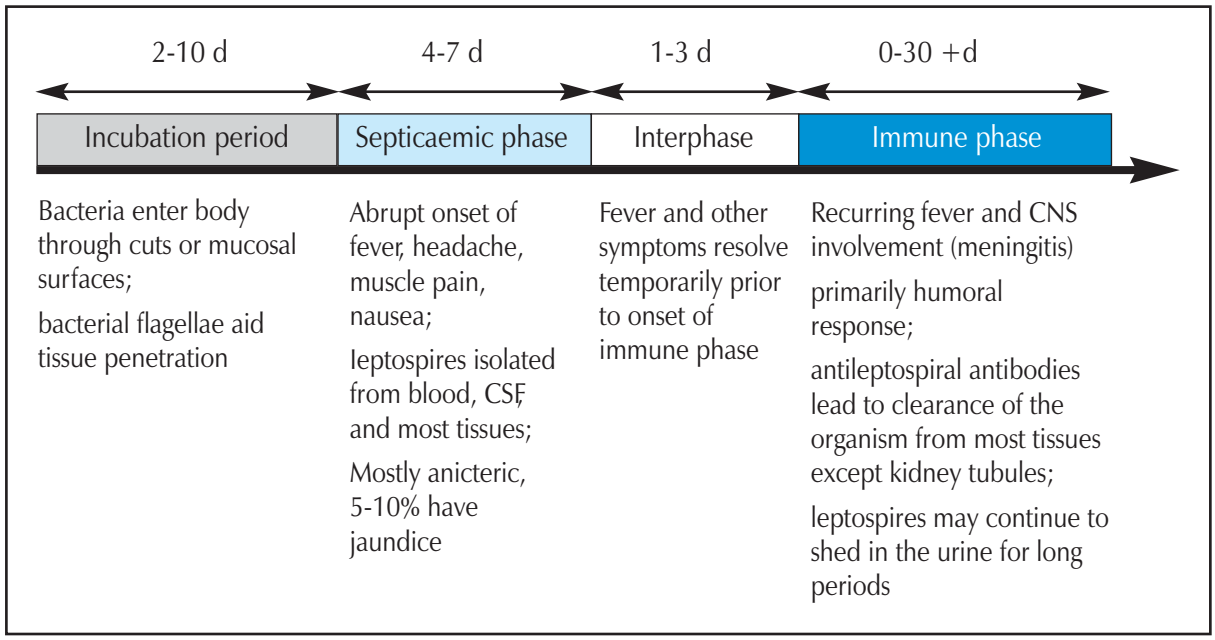
(ii) Weil's syndrome characterized by jaundice, renal failure, haemorrhage and myocarditis with arrhythmias;

(iii) meningitis/meningoencephalitis;

(iv) pulmonary haemorrhage with respiratory failure.

The typical course of leptospirosis with an acute septicaemic phase followed by the immune phase as shown in Figure 1.

**Figure 1: Typical course of leptospirosis**




Courtesy: Dr Richard A. Collins, Hong Kong

## How long is the incubation period?

The incubation period is usually 7–10 days, with a range of 2–30 days.

## When should clinicians consider the diagnosis of leptospirosis?

The diagnosis of leptospirosis should be considered in any patient presenting with an abrupt onset of fever, chills, conjunctival suffusion, headache, myalgia and jaundice. The diagnosis is more difficult when patients present with symptoms of cough, dyspnoea, nausea, vomiting, abdominal pain, diarrhoea, arthralgias and a skin rash.

Conjunctival suffusion and muscle tenderness, most notable in the calf and lumbar areas, are the most distinguishing physical findings.

Suspicion is further increased if there is a history of occupational or recreational exposure to infected animals or to an environment potentially contaminated with animal urine. Once the possibility of leptospirosis has been considered, appropriate diagnostic tests and clinical management should be instituted.

## Why is leptospirosis underdiagnosed?

Leptospirosis may be underdiagnosed because:

(a) the diagnosis is difficult to confirm;

(b) it may be confused with other diseases;

(c) the disease may be mild and not be investigated in the laboratory;

(d) laboratory tests may not be available or the available tests have low sensitivity during early phase of disease as most of the available tests detect antibodies.

## What are the clinical laboratory findings in patients with leptospirosis?

The study of specimens from hospitalized patients has shown various non-diagnostic abnormalities including elevated erythrocyte sedimentation rate, thrombocytopaenia, leucocytosis, hyperbilirubinaemia, elevated serum creatinine, elevated creatinine kinase and elevated serum amylase.

## What causes the pathological phenomena in leptospirosis?

The clinical manifestations are caused by damage to the endothelial lining of small blood vessels by mechanisms that are still poorly understood. All the internal organs may be affected, which explains the wide range of clinical manifestations, e.g. interstitial nephritis and tubular, glomerular and vascular kidney lesions lead to uraemia and oliguria/anuria; vascular injury to hepatic capillaries, in the absence of hepatocellular necrosis, causes jaundice; inflammation of the meninges causes headache, neck stiffness, confusion, psychosis, delirium, etc.; thrombocytopaenia (reduction in the number of blood platelets) may contribute to bleeding.

## What is the case-fatality rate due to leptospirosis?

Case-fatality rates in different parts of the world have been reported to range from <5% to 70%. These figures are not very reliable as in many areas the occurrence of the disease is not well documented. In addition, mild cases may not be diagnosed as leptospirosis.

Major improvements in the prognosis of severe leptospirosis have been made in recent decades, thanks to the use of haemodialysis as a means of supporting the reversible renal failure that may occur in some cases and to aggressive supportive care.

## What is the outcome of leptospirosis during pregnancy?

Leptospirosis during pregnancy may lead to fetal death, abortion, stillbirth or congenital leptospirosis, but only a few such cases have been reported.

# Immunology and diagnostics

## What is the nature of the antibody response?

Humans react to an infection by leptospires by producing specific anti-*Leptospira* antibodies. Seroconversion may occur as early as 3–7 days after the onset of disease but sometimes only after 10 days or longer.

Immunity to leptospirosis is primarily humoral, (i.e. mediated by antibody-producing branch of the immune system), the result of B-cell and Th-2 T-helper (CD4) cell stimulation. Cell-mediated immunity does not appear to be important, but may be responsible for some of the late manifestation of the disease.

IgM class antibodies usually appear somewhat earlier than IgG class antibodies, and generally remain detectable for months or even years but at low titre. Detection of IgG antibodies is more variable. They may sometimes not be detected at all, or be detectable for only relatively short periods of time, but may sometimes persist for several years.

## Is the antibody response protective?

It is generally believed that serovar-specific antibodies are protective and that a patient is immune to reinfection with the same serovar as long as the concentration (titre) of specific antibodies is high enough. Antibodies provoked by an infection with a particular serovar do not necessarily protect against infection with other serovars.

## Why is laboratory support needed for leptospirosis?

Laboratory support is needed:

(1) To confirm the diagnosis. Leptospirosis is difficult to distinguish from a number of other diseases on clinical grounds. Laboratory methods help to confirm leptospirosis where the disease is suspected on clinical grounds.

(2) For epidemiological and public health reasons, namely to determine which serovar caused the infection, the likely source of infection and the potential reservoir and its location. This helps guide control strategies.

## What methods are available for the diagnosis of leptospirosis in the laboratory?

The disease is usually diagnosed in the laboratory by detecting antibodies, (serodiagnosis), by culturing the bacteria from blood, urine or tissues, or by demonstrating the presence of leptospires in tissues using antibodies labelled with fluorescent markers. Other methods may be available in some centres, e.g. the polymerase chain reaction (PCR) and (immuno) staining.

Microscopic agglutination test (MAT) is the gold standard serologic test. High specificity is the major advantage of MAT. This test uses live, in vitro, cultivated leptospires from representative serogroups. MAT is only performed in reference labs and requires acute and convalescent samples for diagnostic confirmation. MAT-detectable antibodies usually do not develop before the end of the first week of illness. Diagnosis of leptospirosis by MAT often requires paired serum samples, which delays the diagnosis. Thus, the operating characteristics of MAT limit its use primarily to retrospective diagnosis.

## What clinical samples should be collected for examination?

This will depend on the phase of the infection. Leptospires usually circulate in the blood of the patient for about 10 days after the onset of the disease. They also appear in other body fluids, such as urine and cerebrospinal fluid, a few days after the onset of disease and penetrate internal organs during this time.

Detectable titres of antibodies appear in the blood about 3–10 days after the onset of disease, but sometimes later, especially if antibiotic treatment is instituted.

The samples that are useful and most commonly collected are therefore:

(1) Blood with heparin (to prevent clotting) for culture in the first 10 days. Blood culture more than 10 days after disease onset is not useful as leptospires have mostly disappeared from the blood and antibodies will have become detectable in the serum allowing serodiagnosis. Samples for culture should be stored and transported at ambient temperatures, since low temperatures are detrimental to pathogenic leptospires. Blood with heparine is not useful for PCR, which preferably requires EDTA blood.

(2) Clotted blood or serum for serology. These should preferably be collected twice at an interval of several days based on the date of onset of disease and the probable time of seroconversion. The testing of paired sera is necessary to detect a rise in titres between the two samples or seroconversion, and thus to confirm the diagnosis of leptospirosis. A negative serological result in the early phase of the disease does not exclude leptospirosis.

(3) Urine for culture. Leptospires die quickly in urine. The use of urine for culture may be of value only when a clean sample can be obtained and inoculated into an appropriate culture medium not more than two hours after voiding. Survival of leptospires in acid urine may be increased by making it neutral.

(4) Postmortem samples. It is important to collect specimens from as many organs as possible, including the brain, cerebrospinal fluid, lungs, kidney, liver, pancreas and heart, as well as heart blood, if possible, for serology, (immuno)staining and PCR .

## What laboratory method is most frequently used to diagnose leptospirosis?

Current methods for the direct detection of leptospires are either slow or of limited reliability so that serology is often the most appropriate diagnostic method. Moreover, in practice, patients often seek medical care or are admitted in hospitals when they have already been ill for a sufficiently long time to have produced detectable antibodies.

Several types of antibody detection assays have been developed for early diagnosis of leptospirosis. These includes the haemolytic test, indirect haemagglutination assay, indirect immunofluorescence, indirect IgM ELISA, IgM dot-ELISA, immobilized antigen dipstick and lateral flow assays. While these methods are much simpler than MAT, they still need a lag period after infection before antibodies become detectable. One should be careful while interpreting the test results due to varying sensitivity and specificity of the test method.

The ELISA tests have been the most readily applicable for the rapid detection and diagnosis of leptospirosis. Several published studies have compared and evaluated a range of commercially available assays.

## Is positive serology proof of a current infection?

Not always. Detection of antibodies is by itself no proof of a current infection as some antibodies may persist for long periods after an infection. Generally, seroconversion (first sample, no detectable titre, second sample,

positive, i.e. above the cut-off point) or a four-fold or higher rise in titre (first sample, low titre, second sample, much higher titre) in consecutive serum samples is considered to be diagnostic proof of recent or current infection.

A high IgM titre, i.e. a titre several-fold above the cut-off point in a single serum specimen as detected by ELISA or a similar test is consistent with current or recent leptospirosis, but it should be remembered that IgM class antibodies may remain detectable for several months or even years.

Serological data are important in the diagnostic process but must always be considered in conjunction with the clinical presentation and epidemiological data (a history of possible exposure, presence of risk factors). Isolation of pathogenic leptospires is the only direct and definitive proof of infection.

## What are the direct methods of detecting leptospires?

Direct methods include culture, dark-field microscopy, (immuno)staining and the polymerase chain reaction (PCR).

## How can leptospires be cultured in media?

Leptospires grow in specific culture media. Direct inoculation of 2-3 drops of blood into culture medium is the ideal method. Their growth is relatively slow, with a doubling time of about 6–8 hours at best. Optimal temperatures for growth are 28 °– 30 °C. Some serovars are, however, more fastidious than others in terms of their requirements in culture.

## Does culture contribute to a quick and early diagnosis?

Unfortunately, leptospires grow slowly so that, by the time they can be identified in the culture, the patient will already have antibodies detectable by serology. For this reason, culture does not contribute to a rapid diagnosis in the early phase of the disease. It is also a relatively insensitive diagnostic method.

## What are the uses of culture?

Isolation of pathogenic leptospires is proof of an infection. Also, isolated leptospires can be typed to identify serovars. Typing of isolated leptospires is useful in the surveillance of local pathogenic serovars, the recognition of new patterns of disease presentation, assessing the effectiveness of intervention measures, etc.

## How is dark-field microscopy used?

Leptospires are observed as thin, coiled, rapidly moving micro organisms in fluids such as culture medium, blood or urine using dark-field microscopy . Leptospires can be concentrated in blood or urine by differential centrifugation.

## What are the advantages and disadvantages of dark-field microscopy?

**Advantage:**

- Dark-field microscopy is particularly useful for observing leptospires in culture, particularly when they are present in large numbers, and for observing agglutination in MAT.

**Disadvantages:**

- Dark-field microscopy is technically demanding. Recognizing leptospires is difficult, particularly when only small numbers are present. Artefacts, such as fibrin threads in blood, are easily mistaken for leptospires.
- False-positive misdiagnosis has frequently occurred. Dark-field microscopy is therefore useful only to those with considerable experience in observing leptospires. Both false-positive and false-negative diagnoses are too easily made. The results of dark-field microscopy of clinical material should always be confirmed by other tests.

## What is PCR for leptospirosis?

PCR is a method of amplifying specific segments of leptospiral DNA, e.g. in clinical samples such as blood, to detectable levels. Thus, the presence of leptospires is confirmed by detecting and identifying specific segments of leptospiral DNA.

## What are the advantages and disadvantages of PCR?

**Advantage:**

- PCR can rapidly confirm the diagnosis in the early phase of the disease, when bacteria may be present and before antibody titres are at detectable levels.

**Disadvantages:**

- PCR requires special equipment and dedicated laboratory space, and also highly skilled personnel.
- Especially conventional PCR may give false-positive results in the presence of minute amounts of extraneous DNA that may contaminate working areas. It may also give false-negative results because inhibitors are present in the clinical materials that are being examined.

The validity of PCR data depends essentially on the quality controls included in the test. Although PCR technology is now widely used for the diagnosis of many diseases, its general value for the rapid diagnosis of leptospirosis has not been evaluated worldwide as it is not yet widely used, particularly in tropical and subtropical countries.

# Clinical case management

## What is the optimal treatment for leptospirosis?

Treatment with effective antibiotics should be initiated as soon as the diagnosis of leptospirosis is suspected and preferably before the fifth day after the onset of illness. The benefit of antibiotics after the fifth day of the disease is controversial. However, most clinicians treat with antibiotics regardless of the date of onset of the illness.

Clinicians should never wait for the results of laboratory tests before starting treatment with antibiotics. This is because serological tests do not become positive until about a week after the onset of illness, and cultures may not become positive for several weeks.

In severe cases, admission to a hospital is necessary. Aggressive supportive care with strict attention to fluid and electrolyte balance is essential. Peritoneal or haemodialysis is indicated in renal failure. Mechanical ventilation is indicated for lung hemorrhagic manifestation. Excellent supportive care and dialysis have reduced the mortality due to this illness in recent years.

## What are the best antibiotics for treating leptospirosis?

Severe cases of leptospirosis should be treated with high doses of intravenous penicillin. Less severe cases can be treated with oral antibiotics such as amoxycillin, ampicillin, doxycycline or erythromycin. Third-generation cephalosporins, such as ceftriaxone and cefotaxime, and quinolone antibiotics also appear to be effective.

# Prevention and control

## How can leptospirosis be prevented and controlled?

Because of the large number of serovars and infection sources and the wide differences in transmission conditions, the control of leptospirosis is complicated and will depend on the local conditions. Control can be achieved by controlling the reservoir or reducing infection in animal reservoir populations such as dogs or livestock. Control of wild animals may be difficult. Preventive measures must be based on knowledge of the groups at particular risk of infection and the local epidemiological factors.

Prevention and control should be targeted at :

(a) the infection source;

(b) the route of transmission between the infection source and the human host; or

(c) infection or disease in the human host.

## How can the infection source be controlled?

It is important to establish what animal species are the infection sources in a particular area. Control measures can then be targeted to the local reservoir species of animals.

Such measures include:

- the reduction of certain animal reservoir populations, e.g. rats;
- the separation of animal reservoirs from human habitations by means of fences and screens;
- the immunization of dogs and livestock;
- the removal of rubbish and keeping areas around human habitations clean;
- encouraging people not to leave food around, especially in recreational areas where rats may be present.

## How can transmission be interrupted?

It is important to be aware of the risk factors for human infection and, if possible, the infection source. Risk of infection is minimized by avoiding contact with animal urine, infected animals or an infected environment. Where appropriate, protective clothing should be worn and wounds covered with waterproof dressings to reduce the chance of infection if exposure is likely, e.g. occupational or recreational exposure.

## How can humans be protected?

Much depends on detailed knowledge of how, where and when humans may become infected in a particular area. One possibility is to increase awareness of the disease among the population, risk groups and health care providers, so that the disease can be recognized and treated as soon as possible. Doxycycline has been reported to give some protection against infection and disease. In certain countries, vaccines for humans are available, but it should be remembered that they may only provoke immune responses to the serovars included in the vaccine.

## How can humans be protected by immunization?

Immunization by means of vaccines seems to provide a certain degree of protection. Vaccines are, in principle, suspensions of killed leptospires. Protection is largely serovar-specific. In areas where many serovars are causing leptospirosis, a vaccine must consist of different serovars matching those circulating locally. In some countries, where many serovars occur, vaccines consist of a mixture of a few of the most prevalent. Protective antibodies are produced only against the serovars present in the particular vaccine used.

Commercial human vaccines have been produced in France and Cuba. However, these vaccines do not induce long-term protection against infection and do not provide cross-protective immunity against heterogenous leptospiral serovars.

## Can animals be immunized?

Dogs, pigs and cattle can be immunized with vaccines consisting of suspensions of killed leptospires. Protection is largely serovar-specific. Immunization may prevent disease but does not always prevent the development of renal carriage.

## Can pathogenic leptospires in the environment be controlled?

Small areas, such as floors, can be cleaned and disinfected, but disinfecting large natural areas such as lakes or rivers is not possible. Leptospires are sensitive to many environmental influences. They are rapidly killed by disinfectants and desiccation. However, leptospires shed in animal urine can survive in the environment for weeks to months under suitable conditions, e.g. in moist soil or surface water with a neutral or slightly alkaline pH.

## Is there a WHO Collaborating Centre for Research and Training on Leptospirosis in the South-East Asia Region?

Yes. Indian Council of Medical Research (ICMR), the Government of India established a National Leptospirosis Reference Center to carry out research on leptospirosis in Port Blair in 1999. It is interesting that the first report of bacteriologically confirmed cases of leptospirosis originated from the Andaman islands in 1931. The World Health Organization designated the National Leptospirosis Reference Centre, Port Blair as a WHO Collaborating Centre for diagnosis, research, reference and training on leptospirosis.

***Acknowledgement***

Special thanks go to Dr Rudy Hartskeerl, President, International Leptospirosis Society and Dr. P Vijayachari, Director, National Leptospirosis Reference Centre, Regional Medical Research Centre, WHO Collaborating Centre for Diagnosis, Research, Reference and Training on Leptospirosis, Port Blair, India for valuable comments and suggestions