WEIL'S SYNDROME - AN EMERGING INFECTIOUS DISEASE

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Summary

Leptospirosis is a worldwide zoonotic infection with a much greater incidence in tropical regions and has now been identified as one of the emerging infectious diseases. The epidemiology of leptospirosis has been modified by changes in animal husbandry, climate, and human behavior. Resurgent interest in leptospirosis has resulted from large outbreaks that have received significant publicity. The development of simpler, rapid assays for diagnosis has been based largely on the recognition that early initiation of antibiotic therapy is important in acute disease but also on the need for assays which can be used more widely. In this review, the complex taxonomy of leptospires, previously based on serology and recently modified by a genotypic classification, is discussed, and the clinical and epidemiological value of molecular diagnosis and typing is also evaluated.

Key words: Leptospirosis, Assays for diagnosis, Role of Pharmacist.

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Introduction

Leptospirosis, an infectious disease that affects humans and animals, is considered the most common zoonosis in the world. Leptospirosis is often referred to as swineherd's disease, swamp fever, or mud fever. The organism enters the body when mucous membranes or abraded skin come in contact with contaminated environmental sources. The infection causes a systemic illness that often leads to renal and hepatic dysfunctionLeptospirosis is caused by pathogenic spiral bacteria that belong to the genus Leptospira, the family Leptospiraceae, and the order Spirochaetales. These spirochetes are finely coiled, thin, motile, obligate, slow-growing anaerobes. Their flagella allow them to burrow into tissue. The genus Leptospira was originally thought to comprise only 2 species: L interrogans, which is pathogenic, and L biflexa, which is saprophytic.The prevalence is higher in males because they tend to be engaged in outdoor work more frequently than females.

History

Leptospirosis is a zoonosis of ubiquitous distribution, caused by infection with pathogenic *Leptospira* species. The spectrum of human disease caused by leptospires is extremely wide, ranging from subclinical infection to a severe syndrome of multiorgan infection with high mortality. This syndrome, icteric leptospirosis with renal failure, was first reported over 100 years ago by Adolf Weil in Heidelberg (5). However, an apparently identical syndrome occurring in sewer workers was described several years earlier (6, 7). Earlier descriptions of diseases that were probably leptospirosis were reviewed recently (8, 9). Leptospirosis was certainly recognized as an occupational hazard of rice harvesting in ancient China, and the Japanese name akiyami, or autumn fever, persists in modern medicine. With hindsight, clear descriptions of leptospiral jaundice can be recognized as having appeared earlier in the 19th century, some years before the description by Weil. It has been suggested that *Leptospira interrogans* serovar icterohaemorrhagiae was introduced to western Europe in the 18th century by westward extension of the range of of *Rattus norvegicus* from Eurasia. The etiology of leptospirosis was demonstrated independently in 1915 in Japan and Germany. In Japan, Inada and Ido detected both spirochetes and specific antibodies in the blood of Japanese miners with infectious jaundice, and two groups of German physicians studied German soldiers afflicted by "French disease" in the trenches of northeast France. Uhlenhuth and Fromme and Hubener and Reiter (10) detected spirochetes in the blood of guinea pigs inoculated with the blood of infected soldiers. Unfortunately, these two groups became so embroiled in arguments over priority that they overlooked the first publications in English and German of papers by Inada's group, whose initial publications predated their own by 8 months. Confirmation of the occurrence of leptospirosis on both sides of the Western Front was obtained rapidly after the publication in Europe of Inada's work (11,12). Given the initial controversy over nomenclature, it is ironic that the organism had first been described almost 10 years before (13). Stimson demonstrated by silver staining the presence of clumps of spirochetes in the kidney tubules of a patient who reportedly died of yellow fever. The spirochetes had hooked ends, and Stimson named them *Spirochaeta interrogans* because of their resemblance to a question mark. Unfortunately, this sentinel observation was overlooked for many years. The importance of occupation as a risk factor was recognized early.

The role of the rat as a source of human infection was discovered in 1917 (14), while the potential for leptospiral disease in dogs was recognized, but clear distinction between canine infection with *L. interrogans* serovars icterohaemorrhagiae and canicola took several years. Leptospirosis in livestock was recognized some years later. Several monographs provide extensive information on the early development of knowledge on leptospirosis .

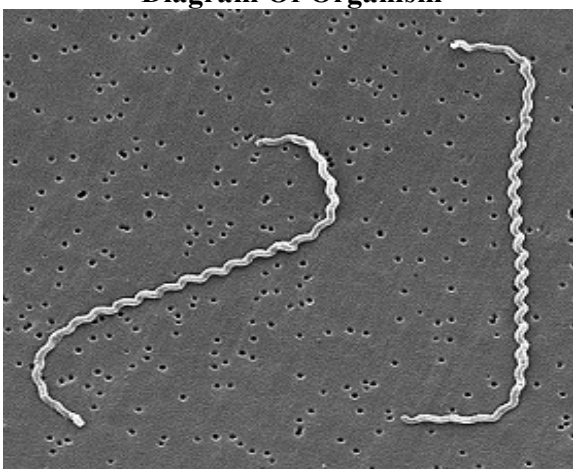
Etiology

Leptospirosis is caused by various species of *Leptospira*, a spirochete in the family Leptospiraceae, order Spirochaetales. The classification of this organism is complex. Before 1989, all of the pathogenic isolates belonged to the species *Leptospira inter-rogans*, which contained more than 200 serovars in 23 serogroups. More recently, the genus *Leptospira* has been reclassified into 16 or more species. Pathogenic serovars are now found in the species *Leptospira interrogans*, *L. noguchii*, *L. santarosai*, *L. meyeri*, *L. borgpetersenii*, *L. kirschneri*, *L. weilii*, *L. inadai*, *L. fainei* and *L. alexanderi.* The new classification system can be confusing because both pathogenic and non-pathogenic serovars and serogroups occur in the same species and a single serovar or serogroup can occur within multiple species. In clinical laboratories, the older serogroup/serovar classification is often still used .

Communability

Direct person-to-person transmission is rare but possible. *Leptospira* organisms are found in the urine during the second (immune) phase of the disease. Most people excrete these bacteria for 60 days or less, but shedding for months or years has been documented. Other routes of transmission are also possible: one infant was infected during breast feeding, and a case of transmission during sexual intercourse was reported.

Diagram Of Organism



Scanning electron micrograph of Sca*L. interrogans* serovar icterohaemorrhagiae Strain

Biology of leptospires

Leptospires are tightly coiled spirochetes, usually 0.1 mm by6 to 0.1 by 20 mm, but occasional cultures may contain muchlonger cells. The helical amplitude is approximately. 1to0.15mm,and the wavelength is approximately 0.5 mm (15). Thecells have pointedends,either or both of which are usuallybent into a distinctive hook (Fig. 1). Twoaxialfilaments(periplasmic flagella) with polar insertions are located in theperiplasmic space (16). The structure of the flagellar proteinsis complex (17). Leptospires exhibit two distinct forms ofmovement, translational and nontranslational (18). Morphologicallyall leptospires are indistinguishable, but the morphologyof individual isolates varies with subculture in vitro and canbe restored by passage in hamsters (19). Leptospires have atypical double membrane structure in common with other spirochetes,in which the cytoplasmic membrane and peptidoglycancell wall are closely associated and are overlain by an outermembrane (20). Leptospiral lipopolysaccharide has a compositionsimilar to that of other gram-negative bacteria (21), buthas lower endotoxic activity (22). Leptospires may be stainedusing carbol fuchsin counterstain (23).Leptospires are obligate aerobes with an optimum growthtemperature of 28 to 30°C. They produce both catalase andoxidase (24). They grow in simple media enriched with vitamins(vitamins B2 and B12 are growth factors), long-chain fattyacids, and ammonium salts (25). Long-chain fatty acids areutilized as the sole carbon source and are metabolized byb-oxidation .

Types of leptospirosis

Anicteric Leptospirosis

The great majority of infections caused by leptospires are either subclinical or of very mild severity, and patients will probably not seek medical attention. A smaller proportion of infections, but the overwhelming majority of the recognized cases, present with a febrile illness of sudden onset. Other symptoms include chills, headache, myalgia, abdominal pain, conjunctival suffusion, and less often a skin rash . If present, the rash is often transient, lasting less than 24 h. This anicteric syndrome usually lasts for about a week, and its resolution coincides with the appearance of antibodies. The fever may be biphasic and may recur after a remission of 3 to 4 days. The headache is often severe, resembling that occurring in dengue, with retro-orbital pain and photophobia. Myalgia affecting the lower back, thighs, and calves is often intense (26). with aseptic meningitis, whereas only 31% of patients aged 15 to 29 years did so and only 10% of those over 30 years of age. Mortality is almost nil in anicteric leptospirosis (27), but death resulting from massive pulmonary hemorrhage occurred in 2.4% of the anicteric patients (28). The differential diagnosis must include common viral infections, such as influenza, human immunodeficiency virus seroconversion (29), and, in the tropics, dengue (30, 31,32), in addition to the bacterial causes of fever of unknown origin, such as typhoid. Turner provided a comprehensive list of other conditions that may be mimicked by leptospirosis, including encephalitis, poliomyelitis, rickettsiosis, glandular fever (infectious mononucleosis), brucellosis, malaria, viral hepatitis, and pneumonitis. Hantavirus infections must also be considered in the differential diagnosis for patients with pulmonary involvement (33). Petechial or purpuric lesions may occur, and recently, cases of leptospirosis resembling viral hemorrhagic fevers have been reported in travelers returning from Africa.

Icteric Leptospirosis

Icteric leptospirosis is a much more severe disease in which the clinical course is often very rapidly progressive. Severe cases often present late in the course of the disease, and this contributes to the high mortality rate, which ranges between 5 and 15%. Between 5 and 10% of all patients with leptospirosis have the icteric form of the disease (34). The jaundice occurring in leptospirosis is not associated with hepatocellular necrosis, and liver function returns to normal after recovery. Serum bilirubin levels may be high, and many weeks may be required for normalization (35). There are moderate rises in transaminase levels, and minor elevation of the alkaline phosphatase level usually occurs. The complications of severe leptospirosis emphasize the multisystemic nature of the disease. Leptospirosis is a common cause of acute renal failure (ARF), which occurs in 16 to 40% of cases (36). A distinction may be made between patients with prerenal azotemia (non-ARF) and those with ARF. Patients with prerenal azotaemia may respond to rehydration, and decisions regarding dialysis can be delayed for up to 72 h (37). In patients with ARF, oliguria (odds ratio [OR], 9.98) was a significant predictor of death. Serum amylase levels are often raised significantly in association with ARF (38), but clinical symptoms of pancreatitis are not a common finding (39,40,41). Necrotizing pancreatitis has been detected at autopsy (42,43). Thrombocytopenia (platelet count of $<100 \times 10^9$/liter) occurs in ≥50% of cases and is a significant predictor for the development of ARF (44). However, thrombocytopenia in leptospirosis is transient and does not result from disseminated intravascular coagulation (45,46). The occurrence of pulmonary symptoms in cases of leptospirosis was first noted by Silverstein (47). Subsequent reports have shown that pulmonary involvement may be the major manifestation of leptospirosis in some clusters of cases (48,49,50,51) and in some sporadic cases (52). The severity of respiratory disease is unrelated to the presence of jaundice (53). Patients may present with a spectrum of symptoms, ranging from cough, dyspnea, and hemoptysis (which may be mild or severe) to adult respiratory distress syndrome (54,55,56,57,58,59). Intra-alveolar hemorrhage was detected in the majority of patients, even in the absence of overt pulmonary symptoms (60). Pulmonary hemorrhage may be severe enough to cause death (61,62,63). The incidence of respiratory involvement varies.Rales are more common in icteric than in nonicteric leptospirosis (64). . Cigarette smoking was reported as a risk factor for the development of zulmonary symptoms (65).Radiography generally reveals diffuse small opacities which may be widely disseminated or which may coalesce into larger areas of consolidation, with increasing severity of symptoms (66,67,68). Pleural effusions may occur (69). The patchy infiltrates which are commonly seen reflect areas of intra-alveolar and interstitial hemorrhage (70). Cardiac involvement in leptospirosis is common but may be underestimated. Fatal myocarditis was first described in 1935. Clinical evidence of myocardial involvement, including abnormal T waves, was detected in 10% of 80 severe icteric cases in Louisiana, while similar electrocardiographic (ECG) abnormalities were detected in over 40% of patients in China, India, Sri Lanka, and the Philippines, including both icteric and nonicteric cases. However, in a prospective study in Malaysia, identical ECG changes were found in patients with either leptospirosis or malaria (71), and it was concluded that such ECG changes were nonspecific. Other ECG abnormalities have been reported less frequently. A mortality rate of 54% was reported in severe leptospirosis cases with myocarditis.

Transmission

Leptospirosis is spread through contact with water,soil,vegetation,orany part of a moist environment contaminated by urine or tissue of infected animals or humans.This bacteria can be inactivated by drying, but can survive in a moist environment for weeks or months.Humans and animals can become infected urine or other body tissue.Leptospira organism can enter the body through broken skin[cut or stracth]or mucous membranes[lining of the mouth,eyes,nose,or genitalia].[Infection can also occur through ingestion of contaminated water or food].If an animal is infected with a serovar or type of leptospirs that is adapted to that species of animal,then the animal will not show clinical signs of ilness,but will excrete the bacteria in its urine for monthsor even years contaminating the environment(72).This serovar adaptation often occurs in rats,mice and wild life raccoon,opossums and skunks.Animals will showclinical signs of leotospirosis when infectedwith aserovarto whichit is notadapted.

Incubation period

The incubation period in humans is usually 7 to 12 days, with a range of 2 to 29 days.

Pathology

The majority of infections are subclinical or extremely mild, however severe infections are possible in all target groups and natural immunity is not expected. Leptospires entering the body migrate to the lymphatic and circulatory systems within minutes, and an extremely rapid systemic infection develops thereafter - however the growth rate of leptospires is slow, therefore the incubation period can be between 2 and 28 days - typically ranging between 3 and 14 days. In all cases the onset of symptoms is very sudden, with a severe and incapacitating headache seen in over 90% of cases. Bilateral conjunctival suffusion, signs of meningism and pyrexia of 39°C or above are expected. A red transient pinprick non-blanching rash is often seen, and patients frequently complain of myalgia, fatigue and nausea. There may be a nonproductive cough but with minor haemoptysis. Psychological changes are often seen, with patients feeling depressed, confused, aggressive and sometimes psychotic - with schizophrenia and hallucinations, personality changes and violence. The bacteria are present in the bloodstream during the first few days (S0 to S9) and so can be directly isolated. After this period the bacteria are confined to the tissues and testing must rely on detection of antibodies.

Uniphase infections

In mild cases the infection follows a single phase, lasting 3 to 7 days, with the above symptoms appearing on day S0, peaking on day S2 and reducing thereafter. It can often therefore be misdiagnosed as one of the multitude of other pyrexic infections. Antibiotics can still be of benefit but patients will recover without intervention.

Biphasic infection

In moderate and severe cases the infection follows two phases, the initial septicaemic phase as above, followed by a transient remission, then a rapid decline one or two days later. Icteric symptoms present during this second phase, and without intervention it usually progresses to

mortality within a short time. Biphasic pathology does not always indicate a severe infection, and indeed in the most virulent cases the distinction is lost, with the patient rapidly deteriorating from S0 and without treatment often dying within ten days.

Diagnostic tests

Leptospirosis can be diagnosed by culture, detection of antigens or nucleic acids, or serology. The location of the organisms varies with the form of the disease. In acute infections, *Leptospira* may be found in the blood, milk, and cerebrospinal, thoracic or peritoneal fluids. During chronic infections, they are sometimes found in the urine. The liver, lung, brain and kidney are collected at necropsy from acute cases, and the kidney and genital tract are tested in chronic cases. Organisms can also be found in the body fluids or tissueof aborted fetuses. *Leptospira* species can be cultured on a variety of mediabut are fastidious and grow slowly on primary isolation.Special transport media may be required for shipment to thelaboratory. Depending on the serovar, culture may take upto 13 to 26 weeks. Identification to the species, serogroup and serovar level is done by reference laboratories, usinggenetic and immunologic techniques. *Leptospira* can also be identified in clinical samples by immunofluorescence and immunhistochemical staining, as well as DNA probesand polymerase chain reaction (PCR) techniques. Darkfieldmicroscopy can be used but is non-specific. Silver stainingis sometimes useful as an adjunct technique. These organismsstain poorly with the Gram stain. Antigen-detection techniques including ELISAs and radioimmunoassay havebeen reported in the literature.Serology is also used for diagnosis. Paired acute and convalescent samples are preferred from most animals, buta single positive sample from an aborted fetus is diagnostic.Herd tests are often used in ruminants. The most commonlyused serological tests are the microscopic agglutination test(MAT) and enzyme-linked immunosorbent assays (ELISAs).Serovar-specific ELISAs are available in veterinary medicine,and cross-reactions are less common in animals thanin humans. Other serologic tests include radioimmunoassay,the microcapsule agglutination test, immunofluorescence,counterimmunoelectrophoresis and thin-layer immunoassay. A milk ELISA can detect antibodies in samples from individualcows or in bulk milk. Titers may become undetectable inchronically infected dogs that are still shedding organisms(73,74,75,76,77).

Preventive measures

Environmental Measures

- To prevent illness, prevent contamination of living, working, and recreational areas by urine of infected animals.
- Control rodent populations in areas of human habitation.
- Domestic animal owners should take necessary precautions to minimize their animal's potential contact with
- wildlife (e.g., do not feed pets outside or allow animals to roam unsupervised).
- Do not allow animals to urinate in or near ponds, pools, or puddles.
- Keep animals away from gardens, playgrounds, sandboxes, and other places children may play.
- Among domesticated animals, vaccination of swine, cattle, and dogs does not provide 100% protection. Vaccines
- are generally effective in preventing symptoms of disease in animals, but do not protect against all serotypes and
- do not protect completely against infection or prevent shedding of organisms in the urine(78,79).

Role of pharmacist in prevention of Weil's syndrome

- *The pharmacists have to advice and* educate the public to prevent leptospirosis, on how the disease is transmitted and the importance of proper food storage and garbage disposal.
- They should also be counseled to minimize their contact with fresh water, mud, and vegetation that might be contaminated with the urine of infected animals.
- If their occupation or recreational activities require such exposure, education on use of personal protective measures (i.e., proper clothing, footwear, and gloves) should be given. Additional preventive measures include:
- Always wash hands thoroughly after touching items potentially soiled by an animal's urine.
- Use a disinfectant cleaning solution or a solution of one part bleach in ten parts water to clean areas or itemssoiled by the animal's urine.

Treatment

Treatment of leptospirosis differs depending on the severity and duration of symptoms at the time of presentation. Patients with mild, flu-like symptoms require only symptomatic treatment but should cautioned to seek further medical help if they develop jaundice. Patients who present with more severe anicteric leptospirosis will require hospital admission and close observation. If the headache is particularly severe, a lumbar puncture usually produces a dramatic improvement.

The management of icteric leptospirosis requires admission of the patient to the intensive care unit initially. Patients with prerenal azotemia can be rehydrated initially while their renal function is observed, but patients in acute renal failure require dialysis as a matter of urgency. This is accomplished satisfactorily by peritoneal dialysis (80,81,82). Cardiac monitoring is also desirable during the first few days after admission (83).

Specific antibiotic treatment was reported soon after penicillin became available, with mixed results (84). Oxytetracycline was also used (85). Early experience was summarized by Alston and Broom in their monograph (86). Few well-designed and well-controlled studies of antibiotic treatment have been reported (87). A major difficulty in assessing the efficacy of antibiotic treatment results from the late presentation of many patients with severe disease, after the leptospires have localized in the tissues.

Doxycycline (100 mg twice a day for 7 days) was shown to reduce the duration and severity of illness in anicteric leptospirosis by an average of 2 days (88). Patients with severe disease were excluded from this study. Two randomized studies of penicillin produced conflicting results. One study included 42 patients with severe leptospirosis, of whom 19 were jaundiced (89); no patient required dialysis and there were no deaths. Intravenous penicillin was given at a dosage of 6 MU/day for 7 days and found to halve the duration of fever. A second study included 79 patients with icteric leptospirosis, of whom 4 died (90). Patients in the treatment group received intravenous penicillin at a dose of 8 MU/day for 5 days. No difference was observed between treatment and control groups in outcome or duration of the illness. There have been no controlled trials of penicillin versus doxycycline for treatment of leptospirosis.

A consistent finding of these studies has been the prevention of leptospiruria or a significant reduction in its duration. This finding alone is sufficient justification for antibiotic use, but any antibiotic treatment should be started as early as possible and should not replace other therapeutic measures. Jarisch-Herxheimer reactions have been reported after penicillin administration (91,92). However, the apparently low risk should not preclude the use of penicillin.

Doxycycline (200 mg orally, once weekly) has been shown to be effective for short-term prophylaxis in high-risk environments (93). Similar findings have been reported in rhesus monkeys challenged experimentally (94). In a recent controlled trial, doxycycline significantly reduced the incidence of clinical disease but not serological evidence of infection. Anecdotal evidence suggests that doxycycline but not penicillin may be used successfully after exposure in laboratory accidents (95). An evidence-based review of antibiotic prophylaxis has been published .

Conclusion

The history and lethal effects of leptospirosis have been understood for many years, and this knowledge has led to the development of effective preventive strategies. In developed countries, leptospirosis continues to be a disease of considerable economic significance in animal husbandry, but the major burden of human disease remains in tropical and subtropical developing countries. The development of several promising approaches to rapid diagnosis has been based largely on the recognition that early initiation of antibiotic therapy is important in acute disease, but also on the need for simpler assays which can be used more widely. However, many of these diagnostic advances will be unavailable to those populations for which they would be most useful. At a more fundamental level, understanding of the mechanisms of pathogenesis remains incomplete, but recent advances in the molecular biology of leptospires offer the prospect of more rapid progress in the future.

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References

1. Palaniappan RU, Ramanujam S, Chang YF. Leptospirosis: pathogenesis, immunity, and diagnosis. Curr Opin Infect Dis. Jun 2007;20(3):284-92. .
2. Chu KM, Rathinam R, Namperumalsamy P. Identification of Leptospira species in the pathogenesis of uveitis and determination of clinical ocular characteristics in south India. J Infect Dis. May 1998;177(5):1314-21
3. Inada R, Ido Y, et al. Etiology, mode of infection and specific therapy of Weil's disease. J Exp Med. 1916;23:377-402.
4. Aston JM, Broom JC. Leptospirosis in Man and Animals. Edinburgh and London,England: Livingstone; 1958.
5. Bajani MD, Ashford DA, Bragg SL, et al. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. J Clin Microbiol. Feb 2003;41(2):803-9.
6. Weil A. Ueber eine eigentümliche, mit Milztumor, Icterus und Nephritis einhergehende akute Infektionskrankheit. Dtsche Arch Klin Med. 1886;39:209–232.

7. Alston J M, Broom J C. Leptospirosis in man and animals. E. & S. Edinburgh, U.K: Livingstone; 1958 Costa S, Troisier J. Un cas de spirochétose ictéro-hémorragique. Bull Mém Soc Méd Hôpitaux de Paris. 1916;40:1635–1639

8. Landouzy L T J. Fièvre bilieuse ou hépatique. Gaz Hôpital. 1883;56:809.

9. Landouzy L T J. Typhus hépatique. Gaz Hôpital. 1883;56:913.

10. Everard J D. Leptospirosis. In: Cox F E G, editor. The Wellcome Trust illustrated history of tropical diseases. The Wellcome Trust, London, U.K. 1996. pp. 111–119. , 416–418.

11. Faine S. *Leptospira* and leptospirosis. Boca Raton, Fla: CRC Press; 1994. 213.

12. Hübener E A, Reiter H. Beiträge zur Aetiologie der Weilschen Krankheit. Dtsch Med Wochenschr. 1915;41:1275–1277.

13. Dawson B, Hume W E. Jaundice of infective origin. Q J Med. 1916;10:90–131

14. Wilmaers L, Renaux E. Quarante-sept cas de spirochétose ictéro-hémorragique. Arch Med Belg. 1917;70:115–207.

15. Stimson A M. Note on an organism found in yellow-fever tissue. Public Health Rep. 1907;22:541.

16. Ido Y, Hoki R, Ito H, Wani H. The rat as a carrier of *Spirochaeta icterohaemorrhagiae*, the causative agent of Weil's disease (spirochaetosis icterohaemorrhagica). J Exp Med. 1917;26:341–353.

17. Weyant R S, Bragg S L, Kaufman A F. *Leptospira* and *Leptonema*. In: Murray P R, Baron E J, Pfaller M A, Tenover F C, Yolken R H, editors. Manual of clinical microbiology. 7th ed. Washington, D.C.: ASM Press; 1999. pp. 739–745

18. Faine S, Adler B, Bolin C, Perolat P. *Leptospira* and leptospirosis. 2nd ed. Melbourne, Australia: MedSci; 1999.

19. Swain R H A. The electron-microscopical anatomy of *Leptospira canicola*. J Pathol Bacteriol. 1957;73:155–158.

20. Trueba G A, Bolin C A, Zuerner R L. Characterization of the periplasmic flagellum

21. Proteins of *Leptospira interrogans*. J Bacteriol. 1992;174:4761–4768.

22. Berg H C, Bromley D B, Charon N W. Leptospiral motility. In: Stanier R Y, Rogers H J, Ward J B, editors. Relations between structure and function in the prokaryotic cell. 28th Symposium of the Society for General Microbiology. Cambridge, U.K: Cambridge University Press; 1978. pp. 285–294.

23. Ellis W A, Hovind-Hougen K, Moller S, Birch-Andresen A. Morphological changes upon subculturing of freshly isolated strains of Leptospira interrogans serovar hardjo. Zentralbl Bakteriol Mikrobiol Hyg A. 1983;255:323–335.

24. Haake D A. Spirochaetal lipoproteins and pathogenesis. Microbiology. 2000;146:1491–1504

25. Vinh T, Adler B, Faine S. Ultrastructure and chemical composition of lipopolysaccharide extracted from Leptospira interrogans serovar copenhageni. J Gen Microbiol. 1986;132:103–109.

26. Shimizu T, Matsusaka E, Takayanagi K, masuzawa T, Iwamoto Y, Morita T, Mifuchi I, Yanagihara Y. Biological activities of lipopolysaccharide-like substance (LLS) extracted from *Leptospira interrogans* serovar *canicola* strain Moulton. Microbiol Immunol. 1987;31:727–735.]

27. Faine S. *Leptospira* and leptospirosis. Boca Raton, Fla: CRC Press; 1994.254.

28. Smibert R M. The Spirochaetales. In: Laskin A I, Lechavelier H A, editors. CRC handbook of microbiology. 2nd ed. Vol. 1. Cleveland, Ohio: CRC Press; 1977. pp. 195–228.

29. Johnson R C, Faine S. *Leptospira*. In: Krieg N R, Holt J G, editors. Bergey's manual of systematic bacteriology. Vol. 1. Baltimore, Md: Williams & Wilkins; 1984. pp. 62–67.

30. Alexander, A. D., A. S. Benenson, R. J. Byrne, R. S. Dı´az-Rivera, L. B. Evans, W. S. Gochenour, H. E. Hall, J. A. Hightower, H. Jeffries, J. deJesu´s, E. Martı´nez, M. Paniagua, J. A. Pons, F. Ramos-Morales, R. Rodrı´-guez-Molina, K. Y. Swisher, T. E. Woodward, and R. H. Yager. 1963.Leptospirosis in Puerto Rico. Zoonoses Res. 2:152–227.

31. Edwards, G. A., and B. M. Domm. 1960. Human leptospirosis. Medicine39:117–156.

32. Wang, C., L. John, T. Chang, W. Cheng, M. Luo, and A. Hung. 1965.Studies on anicteric leptospirosis. I. Clinical manifestations and antibiotictherapy. Chin. Med. J. 84:283–291.

33. Hudson, C. P., P. N. Levett, C. N. Edwards, R. Moosai, and T. C. Roach.1997. Severe primary HIV-1 infection among black persons in Barbados. Int. J. STD AIDS 8:393–397.

34. .Ko, A. I., M. Galvao Reis, C. M. Ribeiro Dourado, W. D. Johnson, L. W.Riley, and the Salvador Leptospirosis Study Group. 1999. Urban epidemicof severe leptospirosis in Brazil. Lancet 354:820–825.

35. .Levett, P. N., S. L. Branch, and C. N. Edwards. 2000. Detection of dengue infection in patients investigated for leptospirosis in Barbados. Am. J. Trop. Med. Hyg. 62:112–114.

36. Sanders, E. J., J. G. Rigau-Perez, H. L. Smits, C. C. Deseda, V. A. Vorndam,T. Aye, R. A. Spiegel, R. S. Weyant, and S. L. Bragg. 1999. Increase ofleptospirosis in dengue-negative patients after a hurricane in Puerto Rico in1996. Am. J. Trop. Med. Hyg. 61:399–404.

37. Antoniadis, A., S. Alexiou-Daniel, L. Fidani, and E. F. K. Bautz. 1995. Comparison of the clinical and serologic diagnosis of haemorrhagic fever with renal syndrome (HFRS) and leptospirosis. Eur. J. Epidemiol. 11:91–115.

38. .Heron, L. G., E. A. Reiss-Levy, T. C. Jacques, D. J. Dickeson, L. D. Smythe, and T. C. Sorrell. 1997. Leptospirosis presenting as a haemorrhagic fever ina traveller from Africa. Med. J. Aust. 167:477–479.

39. Monsuez, J.-J., R. Kidouche, B. Le Gueno, and D. Postic. 1997. Leptospirosispresenting as haemorrhagic fever in visitor to Africa. Lancet 349:254–255.

40. Heath, C. W., A. D. Alexander, and M. M. Galton. 1965. Leptospirosis in theUnited States: 1949–1961. N. Engl. J. Med. 273:857–864, 915–922.

41. Edwards, C. N., G. D. Nicholson, T. A. Hassell, C. O. R. Everard, and J.Callender. 1990. Leptospirosis in Barbados: a clinical study. West Indian Med. J. 39:27–34.

42. Abdulkader, R. C. R. M. 1997. Acute renal failure in leptospirosis. RenalFail. 19:191–198.

43. Ramachandran, S., C. N. A. Rajapakse, M. V. F. Perera, and M. Yoganathan.1976. Patterns of acute renal failure in leptospirosis. J. Trop. Med.

44. Nicholson, G. D., C. N. Edwards, T. A. Hassell, C. O. R. Everard, and J.Callender. 1989. Urinary diagnostic indices in the management of leptospirosis.West Indian Med. J. 38:33–38.

45. Alexander, A. D., A. S. Benenson, R. J. Byrne, R. S. Dı´az-Rivera, L. B. Evans, W. S. Gochenour, H. E. Hall, J. A. Hightower, H. Jeffries, J. de Jesu´s, E. Martı´nez, M. Paniagua, J. A. Pons, F. Ramos-Morales, R. Rodrı´- guez-Molina, K. Y. Swisher, T. E. Woodward, and R. H. Yager. 1963. Leptospirosis in Puerto Rico. Zoonoses Res. 2:152–227.

46. Edwards, C. N. 1999. Leptospirosis and pancreatitis. Pediatr. Infect. Dis. J. 18:399–400.

47. Monno, S., and Y. Mizushima. 1993. Leptospirosis with acute acalculouscholecystitis and pancreatitis. J. Clin. Gastroenterol. 16:52–54.

48. Parenti, D. M., W. Steinberg, and P. Kang. 1996. Infectious causes of acute pancreatitis. Pancreas 13:256–371467. Rajiv, C., R. J. Manjuran, N. Sudhayakumar, and M. Haneef. 1996. Cardiovascular involvement in leptospirosis. Indian Heart J. 48:691–694.

49. Edwards, C. N., and C. O. R. Everard. 1991. Hyperamylasemia and pancreatitisin leptospirosis. Am. J. Gastroenterol. 86:1665–1668.

50. Stolze, E., J. D. G. Schaad, and P. H. Bool. 1959. Weil's disease associated with pancreatic necrosis. Trop. Geogr. Med. 11:93–95.

51. Edwards, C. N., G. D. Nicholson, and C. O. R. Everard. 1982. Thrombocytopenia in leptospirosis. Am. J. Trop. Med. Hyg. 31:827–829.

52. Edwards, C. N., G. D. Nicholson, T. A. Hassell, C. O. R. Everard, and J.Callender. 1986. Thrombocytopenia in leptospirosis: the absence of evidencefor disseminated intravascular coagulation. Am. J. Trop. Med. Hyg.35:352–354.

53. Nicodemo, A. C., M. I. S. Duarte, V. A. F. Alves, C. F. H. Takakura, R. T. M. Santos, and E. L. Nicodemo. 1997. Lung lesions in human leptospirosis:microscopic, immunohistochemical, and ultrastructural features related to thrombocytopenia. Am. J. Trop. Med. Hyg. 56:181–187.

54. Silverstein, C. M. 1953. Pulmonary manifestations of leptospirosis. Radiology 61:327–334.Im, J.-G., K. M. Yeon, M. C. Han, C.-W. Kim, W. R. Webb, J. S. Lee, Y. C.Han, W. H. Chang, and J. G. Chi. 1989. Leptospirosis of the lung: radiographic findings in 58 patients. Am. J. Roentgenol. 152:955–959.

55. Sehgal, S. C., M. V. Murhekar, and A. P. Sugunan. 1995. Outbreak ofleptospirosis with pulmonary involvement in North Andaman. IndianJ. Med. Res. 102:9–12.

56. Wang, C., C. Ch'i, and F. Lu. 1965. Studies on anicteric leptospirosis. III. Roentgenologic observations of pulmonary changes. Chin. Med. J. 84:298–306.

57. Zaki, S. R., W.-J. Shieh, and the Epidemic Working Group. 1996. Leptospirosisassociated with outbreak of acute febrile illness and pulmonaryhaemorrhage, Nicaragua, 1995. Lancet 347:535.

58. Bethlem, N., A. Lemle, and N. G. Pereira. 1991. Leptospirosis. Semin.Respir. Med. 12:58–67.

59. Hill, M. K., and C. V. Sanders. 1997. Leptospiral pneumonia. Semin.Respir. Infect. 12:44–49.

60. Alani, F. S. S., M. P. Mahoney, L. P. Ormerod, P. A. Wright, and M. Garrues. 1993. Leptospirosis presenting as atypical pneumonia, respiratoryfailure and pyogenic meningitis. J. Infect. 27:281–283.

61. Allen, P., S. Raftery, and D. Phelan. 1989. Massive pulmonary haemorrhagedue to leptospirosis. Intensive Care Med. 15:322–324.

62. Berendsen, H. H., J. H. Rommes, B. S. Hylkema, A. F. Meinesz, and H. J.Sluiter. 1984. Adult respiratory failure with leptospirosis. Ann. Intern. Med.101:402.

63. Burke, B. J., J. F. Searle, and D. Mattingly. 1976. Leptospirosis presentingwith profuse haemoptysis. BMJ 2:982.

64. Chee, H. D., G. J. Ossenkoppele, W. Bronsveld, and L. G. Thijs. 1985. Adultrespiratory distress syndrome in Leptospira icterohaemorragiae infection.Intensive Care Med. 11:254–256.

65. De Koning, J., J. G. van der Hoeven, and A. E. Meinders. 1995. Respiratoryfailure in leptospirosis (Weil's disease). Neth. J. Med. 47:224–229.

66. Du Coue¨dic, L., J. P. Courtin, P. Poubeau, B. Tanguy, M. di Francia, andC. Arvin-Berod. 1998. He´morragies intra-alve´olaires patentes et occultes au cours des leptospiroses. Rev. Mal. Respir. 15:61–67.

67. Trevejo, R. T., J. G. Rigau-Perez, D. A. Ashford, E. M. McClure, C. Jarquin- Gonzalez, J. J. Amador, J. O. de los Reyes, A. Gonzalez, S. R. Zaki, W. J. Shieh, R. G. McLean, R. S. Nasci, R. S. Weyant, C. A. Bolin, S. L. Bragg, B. A. Perkins, and R. A. Spiegel. 1998. Epidemic leptospirosis associated with pulmonary hemorrhage—Nicaragua, 1995. J. Infect. Dis. 178:1457–1463.

68. Yersin, C., P. Bovet, F. Me´rien, J. Cle´ment, M. Laille, M. Van Ranst, andP. Perolat. 2000. Pulmonary haemorrhage as a predominant cause of death in leptospirosis in Seychelles. Trans. R. Soc. Trop. Med. Hyg. 94:71–76. 631.

69. Martı´nez Garcı´a, M. A., A. de Diego Damia´, R. Mene´ndez Villanueva, andJ. L. Lo´pez Hontagas. 2000. Pulmonary involvement in leptospirosis. Eur. J. Clin. Microbiol. Infect. Dis. 19:471–474.

70. Lee, R. E. J., S. I. Terry, T. M. Walker, and A. E. Urquhart. 1981. The chest radiograph in leptospirosis in Jamaica. Br. J. Radiol. 54:939–943.

71. .Nery, L. E., A. B. de Paula, J. Nakatani, M. L. dos Santos, and O. R. Ratto. 1977. Clinical, radiological and functional pulmonary manifestations in patients with leptospirosis. Rev. Inst. Med. Trop. Sao Paulo 19:366–373.

72. Teglia, O. F., C. Battagliotti, R. L. Villavicencio, and B. A. Cunha. 1995.Leptospiral pneumonia. Chest 108:874–875.

73. Sodeman, W. A., and J. H. Killough. 1951. The cardiac manifestations ofWeil's disease. Am. J. Trop. Med. Hyg. 31:479–488.

74. Ramachandran, S., and M. V. F. Perera. 1977. Cardiac and pulmonary involvement in leptospirosis. Trans. R. Soc. Trop. Med. Hyg. 71:56–59.

75. Parsons, M. 1965. Electrocardiographic changes in leptospirosis. BMJ2:201–203.

76. Ronald welch,dana dirato,kristy bradley.Canine leptospirosis in okahoma. Okahoma animal disease diagnostic laboratory data 1995-2005.animal health update,vol 8,no 2,july 2001.

77. www.leptospirosis.org.

78. Acha PN, Szyfres B. (Pan American Health Organization [PAHO]). Zoonoses and communicable diseases common to man and animals. Volume 1. Bacterioses and mycoses. 3rd ed. Washington DC: PAHO; 2003. Scientific and Technical Publication No. 580. Leptospirosis; p. 157-168.

79. Aiello SE, Mays A, editors. The Merck veterinary manual. 8th ed. Whitehouse Station, NJ: Merck and Co.; 1998. Leptospirosis; p. 474-477.

80. .Berkow R, Fletcher AJ, editors. The Merck manual. 16th ed. Rahway, NJ: Merck and Co.; 1992. Leptospirosis;p. 153-154.

81. Green-MacKenzie J. Leptospirosis [online]. eMedicine; 2001 Aug. Available at: http://www.emedicine.com/ emerg/topic856.htm.

82. Johnson RC. Leptospirosis [monograph online]. In Baron S, editor. *Medical Microbiology*. 4th ed. NewYork:Churchill Livingstone; 1996.Available at: http://www.gsbs.utmb.edu/microbook/ch035.htm.

83. Acha P.N., Szyfres B. Zoonoses and Communicable Diseases Common to Man and Animals, 3rd Edition Vol. I, Bacterioses and Mycoses. Pan American Health Organization (PAHO). Scientific and Technical Publication 580, 2003.

84. American Academy of Pediatrics. [Leptospirosis.] In: Pickering L.K., ed. Red Book: 2003 Report of the Committee on Infectious Diseases, 26th Edition. Elk Grove Village, IL, American Academy of Pediatrics; 2003: 403–405.

85. Grell G, Ho-Ping-Kong H, Ragbeer M M S, Barrow O, Ward E E, Alleyne G A O. Peritoneal dialysis in severe leptospiral renal failure. West Indian Med J. 1971;20:76–82.

86. .Muthusethupati M A, Shivakumar S. Acute renal failure due to leptospirosis. J Assoc Phys India. 1987;35:631–633.

87. Tan D S K, Suleiman A B, Jeyaindran S. 16 cases of acute renal failure due to leptospirosis. Med J Malaysia. 1986;41:152–155.

88. Dupont H, Dupont-Perdrizet D, Perie J L, Zehner-Hansen S, Jarrige B, Daijardin J B. Leptospirosis: prognostic factors associated with mortality. Clin Infect Dis. 1997;25:720–724.

89. Baber M D, Stuart R D. Leptospirosis canicola: a case treated with penicillin. Lancet. 1946;ii:594–596.

90. Russell R W R. Treatment of leptospirosis with oxytetracycline. Lancet. 1958;ii:1143–1145.

91. Alston J M, Broom J C. Leptospirosis in man and animals. E. & S. Edinburgh, U.K: Livingstone; 1958.

92. Guidugli F, Castro A A, Atallah A N. Antibiotics for treating leptospirosis (Cochrane Review), Cochrane Library, Issue 2. Oxford, U.K: Update Software; 2000.

93. McClain J B L, Ballou W R, Harrison S M, Steinweg D L. Doxycycline therapy for leptospirosis. Ann Intern Med. 1984;100:696–698.

94. Watt G, Padre L P, Tuazon M L, Calubaquib C, Santiago E, Ranoa C P, Laughlin L W. Placebo-controlled trial of intravenous penicillin for severe and late leptospirosis. Lancet. 1988;i:433–435.

95. Edwards C N, Nicholson G D, Hassell T A, Everard C O R, Callender J. Penicillin therapy in icteric leptospirosis. Am J Trop Med Hyg. 1988;39:388–390.