14

Leptospira and Leptospirosis

LEPTOSPIRAS are quite distinct from the other bacteria discussed in chapters 12 through 18 in that they are not normally transmitted from person to person. They infect rodents and other animals and occasionally infect man when he comes into contact with infected animal urine. Leptospiras are included here because sewer workers are exceptionally exposed to the risk of leptospirosis.

Description of Pathogen and Disease

Leptospirosis can be a severe illness, but rarely is it sufficiently common to constitute a major public health problem. Good accounts of leptospirosis have been published by Alston and Broom (1958) and Turner (1967, 1968, and 1970).

Identification

Leptospirosis is an infection with bacteria of the genus *Leptospira* that may take several forms. One of these forms is Weil's syndrome, which develops in about 40 percent of cases of infection by the more virulent serotypes, notably *L. icterohaemorrhagiae*.[1] It is a severe illness with jaundice, neck stiffness, hepatomegaly, sometimes splenomegaly, hemorrhages in the eyes and skin, albuminuria, and hematuria. It has a high fatality rate unless diagnosed and treated in the early stages. However, milder forms of illness can also result from infection by *L. icterohaemorrhagiae* as well as by the many other serotypes (over 100) that are pathogenic to man. In such cases the disease cannot be diagnosed clinically with accuracy because of its protean nature. Its signs and symptoms may mimic many other illnesses such as influenza, septic meningitis, Q-fever, enteric-like illnesses, glandular fever, and brucellosis. In tropical countries it has to be differentiated from malaria, scrub-typhus, dengue, and sand fly fevers. Such illnesses can only be diagnosed as leptospirosis by laboratory tests—isolation of the organisms in blood culture and serological tests that show a rise in the specific antibody levels.

1. See the subsection "Infectious agent," below, for a note on taxonomic nomenclature.

Occurrence

Leptospiras exist in most countries of the world, but whereas certain serotypes (for example, *icterohaemorrhagiae* and *canicola*) are widespread, others tend to be restricted only to certain regions, where their natural (reservoir) hosts are found. In most tropical areas multiple serotypes are circulating.

Although it appears that the incidence of human leptospirosis is low compared with that of other infectious diseases and that cases tend to be sporadic rather than epidemic, it is likely that in certain parts of the world many cases are being missed because they are misdiagnosed and leptospirosis is not being looked for.

At certain times and under certain circumstances, leptospirosis may become a major problem, as it was among the British forces stationed in the jungles and swamps of Malaya and Burma, and as it still is among rice field and sugarcane plantation workers.

Infectious agent

Bacteria of the genus *Leptospira* are essentially parasites of animals, notably rodents—rats, mice, voles, and the like. Leptospiras are slender, flexuous, spiral organisms that are actively motile by means of internal axial filaments analogous to the external flagella of Gram-negative bacilli. They are approximately 0.1 micrometers in width and 6–20 micrometers in length, with bent or hooked ends (see figure 14-1). Some of them are free-living, nonpathogenic, common in fresh water; others are parasites of animals and potential pathogens of man. Leptospiras are

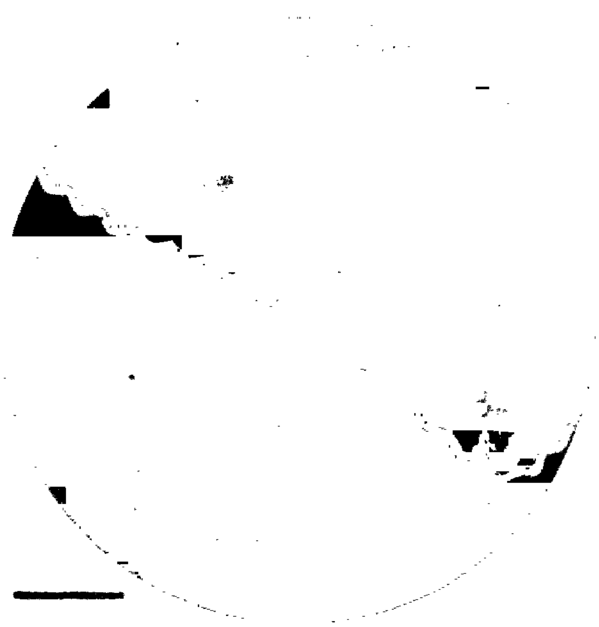

Figure 14-1. Leptospira *under scanning electronmicroscopy*. The organism is spiral and has bent or hooked ends. Scale bar = 1 micrometer. (Photo: J. D. Fulton and D. F. Spooner, National Institute for Medical Research, London, UK)

strictly aerobic. They require animal serum or a serum derivative for growth. The optimum temperature for growth is 28–30°C.

Approximately 150 serotypes of pathogenic leptospiras are recognized. Before 1967 these serotypes were considered as separate species. Recently the antigenically distinct types have been classified as serovarieties (or serotypes) of a single species, *Leptospira interrogans*. For example, an organism referred to as *L. canicola* in the older literature would now be called *L. interrogans* serovar (serotype) *canicola*. Free-living saprophytic leptospiras are now classified as serovars (serotypes) of the species *L. biflexa* and are commonly isolated from surface waters, sediments, and soils (Henry and Johnson 1978).

The first pathogenic serotype to be isolated from human leptospirosis was named *icterohaemorrhagiae*. It is one of the more virulent of the leptospiral serotypes and is the main cause (but not the only cause) of the severe form of the disease, Weil's syndrome. Many different serotypes pathogenic to man and animals have since been reported.

Reservoirs

Each serotype of *Leptospira* has its own preferred host (mainly rodents) in which it exists as a commensal bacterium without causing any apparent harmful effects. It colonizes the kidney tubules, from which it is periodically washed out in the urine.

The rat, *Rattus norvegicus* (brown rat, sewer rat), is the reservoir host of the *icterohaemorrhagiae* serotype, and any environment where these rats are found is a potential hazard to the men who may work there, should they handle material contaminated with rat urine. Persons who have been infected with *icterohaemorrhagiae* include fish workers, butchers, refuse collectors, bricklayers, dock laborers, factory workers, miners, sewer workers, and many others. Any environment that encourages the proliferation of rats is potentially a source of infection with *icterohaemorrhagiae*. Domestic animals may also be infected by this serotype, and they transmit the infection to each other and to the people who look after them.

Transmission

Provided that the external conditions are favorable (moist, relatively warm, shaded from the ultraviolet light of the sun, not salty, and of a neutral pH), leptospiras can remain viable for a time outside the animal body and are then transmissible to other hosts, including man or his domestic animals. The organisms gain access to the body through its contact with the urine-contaminated environment by way of cuts and abrasions of the skin or by way of the mucous membranes of the nose and mouth during immersion in water containing leptospiras. If the new, accidentally infected host is a human being or an animal of a different species than the reservoir host, a disease state may result. In the convalescent stage of the infection, man and these animals may become urinary excretors of leptospiras although, unlike the reservoir hosts, they tend to be temporary rather than chronic shedders. Dogs, cattle (including milking cows), pigs, and horses have all been incriminated in the spread of the disease via their urine. Man-to-man transmission is rare and usually limited to direct contact (for example, by venereal or transplacental routes), but an exceptional case from Vietnam has been reported (Spinu and others 1963) and is discussed below.

Although it is not possible to determine the infective dose of virulent leptospiras in man, experiments on guinea pigs have indicated that the *icterohaemorrhagiae* serotypes (derived directly from an infected guinea pig) may have a lethal dose, and consequently an infective dose, as low as 1 organism.

Incubation period

Symptoms develop after a period ranging from 2 to 14 days, with an average of 7–8 days.

Period of communicability

The length of time a patient may be considered to be capable of communicating leptospiras to other individuals within his environment depends on the time he carries the organism in the kidney tubules. Unlike some animal hosts that may remain carriers for the whole of their life span, humans rarely shed leptospiras in their urine for longer than 4–6 weeks from the onset of infection (see, for instance, Ido and others 1917).

Resistance

All known vertebrate animals are susceptible to leptospiral infection, and there is no known example of natural resistance. Acquired immunity may help to control the incidence of clinical disease in communities living and working in close proximity to sources of infection. This applies especially to rural communities.

Epidemiology

Many of the accounts of leptospirosis record outbreaks of infection among groups of workers in developed countries who have been occupationally exposed to animal urine. For instance, Fairley (1934) made the first report of a fatal case of leptospiral jaundice in a sewer worker in Britain. The patient had been working in a London sewer for only 3 weeks before becoming infected. A serological investigation revealed that eight other sewer workers who had suffered from jaundice had also been infected by the same leptospiral serotype (*icterohaemorrhagiae*), and five other cases of leptospiral jaundice in London sewer workers were subsequently diagnosed. Alston (1935) described three of them. The first was a man, age 52, employed intermittently for 5 years as a flusher; the second was a man 35 years old who had been employed for 3 years, also as a flusher; the third, a fatal case, was a man 52 years old employed relaying sewers. He worked in old open trenches and in sewers that contained much slime and silt. He cut a finger on a broken drain pipe. The wound was dressed, and he went on working until his illness occurred about 2 weeks later. An investigation into the length of service of the men involved in these cases showed that it was not only recent recruits who were liable to infection. It occurred in men who had worked in sewers for a few months to 5 years or more. Infection occurred among those who worked as building laborers as well as among sewer flushers. Laborers who chiseled out and handled slime-covered brickwork in sewers were in direct contact with leptospiras subsequently shown to be present in the slime. These men frequently sustained abrasions and cuts on the hands. The flushers were less likely to sustain abrasions of the skin. They may, however, have become infected through the mucous membranes of the alimentary or respiratory tract by touching the mouth and nose during work. Virulent strains of *icterohaemorrhagiae* were isolated from sewer rats, caught in sewers in eight different parts of London, and also from slime taken from the floor of the sewer near where the first patient (the fatal case) had been working and from the outlet of a house drain into another sewer. The isolation of virulent leptospiras from sewers recalls the isolation of *icterohaemorrhagiae* serotype from slime in a coal mine recorded in 1927 (Buchanan 1927). Johnson, Brown and Derrick (1937) recorded the first three cases of classical Weil's disease in Australia.

Cabelli (1978) stated that leptospirosis has been reported with increasing frequency in the USA over the past five decades and that many cases are no longer related to occupational exposure but to contact with soil or water contaminated by urine. Swimming or wading in small ponds or creeks, recently used by cattle or receiving run-off from nearby pastures, is a common setting for infection. In 1975, 119 cases of leptospirosis were reported in the USA and 36 (29 percent) were attributed to contact with water containing cattle urine. Diesch and McCullouch (1966) isolated serotype *pomona* from bathing water implicated in an outbreak of 15 human cases of *pomona* leptospirosis in Iowa (USA) in 1964; these authors induced leptospiral infections by inoculating guinea pigs with the suspect water (see also Gillespie and Ryno 1963).

In 1977, 86 cases of leptospirosis were reported in the USA, of which 69 percent were males, and there were four deaths. The most probable sources of infection were livestock, domestic pets, and contaminated water. Only 26 percent of cases could be linked to particular vocational activities; of these, farmers were at the greatest risk (Centers for Disease Control 1979). The association between leptospirosis and farming in developed countries is further highlighted by the fact that 42 percent of leptospirosis cases in the UK in 1979–80 were farmers (Coghlan 1981).

Detailed accounts of leptospirosis epidemiology in developing countries are few. The pattern of infection normally depends upon the interaction between man and infected animals in a particular environment. Willis and Wannan (1966) found leptospiral antibodies in the blood of between 31 and 79 percent of people in six villages in different parts of Papua New Guinea. Antibody prevalence rose with age and was similar in males and females. Antibodies, to the same leptospiral serogroups as were found in humans, were also found in rats, dogs, and pigs. The authors noted that in Papua New Guinea the daily pattern of village life brings most individuals into close contact with these animals. Thus, high antibody prevalence is not associated with a particular sex or occupation. By contrast, Damude and others (1979) found that 43 percent of sanitation workers and 39 percent of sugarcane workers in Barbados had antibodies to leptospiras and that this antibody prevalence was significantly higher than that for all other occupations studied. Further, 74 percent of the 215 reported cases of human leptospirosis occurring on Barbados during 1968–74 were males. Caldas and Sampaio (1979) reported on leptospirosis in Salvador (Bahia, Brazil) during 1975. The highest incidence occurred during the rainy season, especially April and May, and possible sources of infection were sewage, rats, water, dogs, mud, and garbage—in that order of frequency.

The operation of foreign armies in tropical areas has stimulated important research on a number of tropical infections for over a century. The study of leptospirosis in Malaysia during the 1950s and 1960s is a relatively recent case in point (Alexander and others 1975; Baker 1965; Baker and Baker 1970; Gordon Smith and others 1961; McCrumb and others 1957). About 40 percent of nonmalarial fever among foreign troops was due to leptospirosis. Infection was strongly associated with jungle maneuvers, and an average case rate of about 1 per battalion per day of jungle duty was reported. The primary rain forests of Malaysia were found to be a hyperendemic focus of leptospirosis. Infection of wild and domestic animals was common, and rats were implicated as the reservoir of greatest importance in the epidemiology of human infection. Pathogenic leptospiras (serotypes of *L. interrogans*) were readily isolated from stagnant and swamp waters in the jungle and also from streams, especially following periods of rain; soil near the stream banks, presumably contaminated by animal urine, frequently contained pathogenic leptospiras, and it was thought that these were being washed into the streams during rain.

A report from Vietnam (Spinu and others 1963) tells a different and unusual story. In Vietnam, as elsewhere, cases of human leptospirosis tend to occur sporadically, usually on the plains among workers in the flooded rice fields, where there is a great diversity of rodent species as well as domestic animals to act as the reservoir hosts for the various serovars that are responsible for human infection. During 1959, however, an epidemic amounting to 121 cases occurred among 240 soldiers working in the jungle territory of northeast Vietnam. Two groups of 80 soldiers were employed in two different forest sites, cutting down trees and bringing them down to the beds of several streams, where a third group of 80 men with a team of buffaloes had the task of dragging the logs downstream and across marshland to the place where the logs were stacked before being loaded onto lorries for further transport. Cases of leptospirosis occurred among soldiers in this third group and among men from the other two teams who were drafted to do the same work. No cases developed among the soldiers felling trees. The pH of the mud and water was 7–7.2, and the atmospheric shade temperature 22–26°C—conditions favorable to the survival of leptospiras. Leptospiras inoculated into samples of water taken from the streams remained viable in the laboratory for 2–5 days. There was no evidence that either the buffaloes or rodents in the area were carriers of the infecting strains of *Leptospira*. The inability of the investigators to find an animal reservoir of infection led to the conclusion that the epidemic had been spread by person-to-person transmission. Soldiers urinated directly onto the waterlogged track down which the logs were dragged and around the loading platforms where the ground was equally boggy. The soldiers, clad only in shorts, worked barefoot and were liable to sustain scratches and abrasions of the skin, and these small wounds allowed the ready entry of leptospiras into the body. The prolonged period of leptospiruria (up to 97 days) shown by some of the patients was thought to be due to the near normal pH of the urine (pH 6.2–7.2), a result of their predominantly vegetarian diet.

Control Measures

Both individual and environmental approaches to leptospirosis control are employed, although in general environmental approaches are more effective.

Individual

Persons who are known to have been at risk through contact with material contaminated with the urine of infected animals should be given a course of penicillin, 2 mega-units per day for 5 days, administered intramuscularly. No special prophylactic drugs are available for leptospirosis. Avoidance of contact with any material containing animal urine (especially rat urine) will greatly reduce the risk of infection. Cuts and abrasions should be covered and protective clothing worn.

In high-risk occupations, vaccination has proved to be effective. It is necessary to prepare the vaccine from strains prevalent in the area, since the level of cross-protection afforded by one serotype may not be adequate for another. Undesirable side effects have been reported, but these have been minimized by the use of vaccines prepared by methods similar to that of Babudieri (1962).

Environmental

To avoid direct transmission of leptospiras contained in animal urine, working premises should be made rat-proof: refuse and food waste should not be left lying around to attract rodents, dogs, foxes, and other scavengers. Chlorination of water supplies will prevent transmission by this route.

Human urine is an uncommon source of infection because prevalence of human infection is relatively low and leptospiras do not multiply or survive for long in urine. Leptospirosis is not an intestinal infection, and the organisms are not likely to be present in the feces. Workers in occupations likely to bring them into contact with human or animal urine should be encouraged to wear protective clothing, rubber boots, and gloves. This applies especially to anyone employed to remove night soil, especially urine, manually.

Occurrence and Survival in the Environment

Leptospiras that infect animals may be found in the environment where animal urine is present, especially in water, mud, slime, or soil.

In water and sewage

Chang, Buckingham and Taylor (1948) found that the survival of *L. icterohaemorrhagiae* in water was heavily dependent on the temperature and the level of bacterial contamination. In river water, the leptospiras survived for 8–9 days at 5–6°C, but at 20–27°C they survived for only 5–6 days, and at 31–32°C their life span was reduced to 3–4 days. In tap water containing 10 per cent human sewage they survived for 6–7 days at 5–6°C, 3–4 days at 25–27°C, and 2–3 days at 31–32°C. At atmospheric temperature, survival times were 18–20 days in sterile tap water, 10–12 days in tap water with added mixed bacterial flora, and only 12–14 hours in undiluted sewage. When the sewage was aerated, however, the leptospiras survived for 2–3 days, indicating that the adverse effect of heavy bacterial growth may be due to anaerobic conditions and to a lowered pH value.

Chang, Buckingham and Taylor (1948) also found that survival in water was greatly reduced by high or low pH and by salinity. In tap water without bacterial contamination, the leptospiras remained viable for over 4 weeks at neutral pH, provided that some nutrients were present. At pH 5 the survival time was reduced to less than 2 days. High pH values were also detrimental. Leptospiras survived for only 18–20 hours in seawater. The hostility of salinity to leptospiras is confirmed by the work of Jamieson, Madri and Claus (1976). At a range of salinities (0.5, 2.0, and 3.5 percent) and temperatures (4, 25, and 37°C), serotype *pomona* survived for less than 24 hours.

The addition of nutrients to sterile water enhances the survival of leptospiras. In sterile tap water plus 1 percent tryptose, survival was up to 50 days, whereas the addition of 0.1 percent horse serum increased the survival time still further to 102 days. However, in the presence of other bacteria the addition of nutrients had the opposite effect. By favoring the multiplication of the other bacteria, addition of nutrient reduced the survival of the leptospiras to approximately 40 hours (Chang, Buckingham and Taylor 1948).

Spinu and others (1963) reported that leptospiras survived for 2–5 days in stream water at 22–26°C. Diesch (1971) recorded a 3-day survival period for leptospiras in stream water and well water.

Noguchi (1918) reported that in unpolluted water, such as drinking water, leptospiras (serotype *icterohaemorrhagiae*) did not remain infectious for longer than one week. Further experiments proved that leptospiras will not grow or survive for long in highly contaminated water, such as polluted river water, sewage, or stagnant cesspools. They invariably disappeared in 48 hours. Noguchi tested the survival time of *icterohaemorrhagiae* in culture medium to which various species of aerobic bacteria had been added. He showed that bacilli of intestinal origin were extremely antagonistic to the growth of the leptospiras.

In summary (see also the appendixes of Feachem and others 1980), leptospiras in clean, sterile water at cool temperatures may survive for up to 20 days, and they may grow and survive for 100 days in the presence of suitable nutrients. However, in water with a rich bacterial flora and at warm temperatures, precisely the environment in which leptospiras are likely to be found after being shed from an infected animal in the tropics, survival times are probably between 1 and 5 days.

In urine

Leptospiras are shed in the urine, and therefore survival in urine is an important determinant of the risk of new infections, especially in excreta disposal systems such as the Vietnamese double-vault latrine, which disposes of urine separately from feces. Noguchi (1918) added serotype *icterohaemorrhagiae* to normal human urine and found that it survived for less than 24 hours due to the acidity. When the urine was neutralized or made slightly alkaline they survived for 24 hours but not for 48 hours. However, when nutrients were added in the form of rabbit serum plasma to neutral or slightly alkaline urine, growth occurred for approximately 10 days. Highly alkaline conditions were as detrimental to leptospiras as was acidity.

In feces and night soil

Leptospiras are not passed in the feces of infected animals but will be mixed with feces in most excreta disposal systems in which urine and feces are treated together. Noguchi (1918) showed that leptospiras would not survive for longer than 24 hours in an emulsion of feces, either normal or from jaundiced patients, with or without added nutrients. This was thought to be due to bacterial contaminants that outgrew the leptospiras, since in sterile fecal material with added nutrients leptospiras survived and remained virulent to guinea pigs for 4 days. Diesch (1971) found that serotype *pomona* survived for up to 5 days in liquid cattle manure.

In soil

Noguchi (1918) added leptospiras to samples of soil rich in organic matter and neutral in pH. The organisms were not detected after 72 hours because of bacterial overgrowth. The more vigorous the growth of bacteria, the less able were the leptospiras to survive.

Karaseva, Chernukha and Piskunova (1973) contaminated measured areas of soil with urine of voles shown to be carriers of leptospiras belonging to serogroups Grippotyphosa or Hebdomadis. It was found that the shortest survival time occurred in areas where the moisture content was low (9.5–16.5 percent), where there was little shade, and where the pH was 5.5. Under these conditions the leptospiras survived for only 6–12 hours. In marshy areas where the moisture content was high (40–60 percent) and the pH 6.9–7.4, leptospiras were seen actively motile for 4–7 days. In deep shade provided by reeds on the shore of a lake, the survival time was 15 days. In one experiment a leptospirosis-free vole was infected with Grippotyphosa serogroup by inoculation with washings from soil contaminated with infected vole urine 5 days before. Thus, the survival time of pathogenic leptospiras in soil under favorable conditions was 15 days, with preservation of their pathogenic properties for at least 5 days.

Inactivation by Sewage Treatment Processes

Chang, Buckingham and Taylor (1948) conducted experiments to determine the resistance of leptospiras to halogen compounds and synthetic detergents and showed that leptospiras are more sensitive to chlorine than are the enteric bacteria. Synthetic detergents that are cationic were shown to be highly leptospiricidal, whereas the anionic detergents were inactive except at high dosage.

Diesch (1971) seeded a laboratory model oxidation ditch with a virulent strain of serotype *pomona* to simulate the shedding of leptospiras by beef cattle. Leptospiras were recovered for at least 61 days. Diesch concluded that an oxidation ditch containing cattle manure is an adequate environment for the survival of leptospiras. If sludge or effluent from such a ditch is used to fertilize land, or if effluents are discharged to rivers, there is clearly a risk of leptospiral transmission to men and other animals.

Diesch (1971) also reported a 5-day survival in the effluent, and a 4-day survival in the sludge, from a cattle manure settling chamber. When compared with the 61-day survival time in the oxidation ditch, these results highlight the importance of oxygen to the survival of leptospiras. Similarly, McGarry and Stainforth (1978) reported Chinese data that leptospiras survive for less than 30 hours in the rich anaerobic fecal liquor of a biogas plant.

Processes with short retention times and involving aeration (for example, trickling filters, activated sludge, oxidation ditches) cannot guarantee the destruction of leptospiras. However, if sewage is held for a week or more, as in waste stabilization lagoons, leptospiras will not survive. Any anaerobic process, such as a septic tank, will also rapidly destroy leptospiras.

Inactivation by Night Soil and Sludge Treatment Processes

Most night soil and sludge treatment processes are anaerobic and will rapidly destroy leptospiras. Diesch (1971) reported a 4-day survival for serotype *pomona* in a cattle manure sludge. However, under many anaerobic conditions survival times are unlikely to exceed 2 days, and there may well be differences among the survival characteristics of the different serotypes (for instance, serotype *pomona* may survive for longer than *icterohaemorrhagiae*).

Leptospiras are sensitive to heat and will be rapidly inactivated by thermophilic sludge treatment processes. Chang, Buckingham and Taylor (1948) recorded the following thermal death points in distilled water: 30 minutes at 45°C, 10 minutes at 50°C, 10 seconds at 60°C, and <10 seconds at 70°C.

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