8. LEPTOSPIROSIS

Leptospiroses are found in animals all over the globe. The leptospires are excreted primarily in the urine; this gives a particular hallmark to their transmission. Their presence has often been detected through infection of man, who forms an exceptional but important link in the chain of transmission.

The animal reservoirs are numerous. Rodents, both domestic and wild, have the primary role. They are true carriers, as they are clinically symptomless chronic excreters of the pathogen. Occasional carriers close to man – dogs, cattle, sheep, goats, and horses – and a wide range of wild animals (birds, reptiles, amphibians, and fish) complete the picture.

Contamination results from the excretion of leptospires primarily through the urine into water, and secondarily into the ground.

As a result, leptospirosis is associated with certain occupations or amusements involving an aquatic environment. The groups most exposed to contamination are workers in alluvial mining operations, rice planters, shepherds, cowherds and swineherds, veterinarians, fish breeders, fishermen, those who practise water sports, and campers.

The leptospire's survival in the outside environment is dependent on some specific environmental conditions (temperature, pH, salinity), the geology of the soil, and particularly the presence of heavy metals as copper.

The genus Leptospira is divided into proven or potential pathogens, classified in the group of L. interrogans, *and non-pathogens (primarily water saprophytes), which are classified with* L. biflexa. *For lack of morphological, metabolic, or culturing criteria, the different varieties making up the species are separated, on the basis of immunological criteria, into more than 180 serotypes or serovars classified into 19 serogroups.*

There is no constant relationship between serotypes and animal reservoirs; each animal species is sensitive to various leptospires. Some associations can be linked to more frequent ecological circumstances. This is the case for L. canicola *and dogs;* L. icterohaemorrhagiae *linked to rats;* L. pomona *to swine and cattle;* L. grippotyphosa *and* Microtus arvalis, *etc.*

Precise diagnosis is not aided by identification based on classical descriptions in textbooks, which focus attention on Weil's relapsing jaundice. In leptospirosis, as in most infectious diseases, the syndrome is neither unique nor uniform, but ranges from hidden, subclinical forms and an influenza-like form, to serous meningitis and serious hepatonephritis, which can degenerate into a major haemorrhagic syndrome.

The diagnosis, based ideally on the isolation and identification of the leptospires, usually relies on serological tests showing significantly elevated antibody levels. Therapy is palliative rather than specific.

The recommended preventive methods are unrealistic. In any event, leptospirosis belongs to the class of non-priority zoonoses in public health.

HISTORICAL BACKGROUND

The key to one of the mysteries of fevers was found by *Weil* in 1886, when he identified a spirochaete-associated fever. The causal bacterium was isolated 30 years later by *Inada* and co-workers (1916), although the role of rodents was established almost simultaneously in Japan. *Noguchi* separated the genus *Leptospira* from *Spirochaeta* in 1917.

Uhlenhuth and *Zuelzer* isolated some aquatic leptospires in 1921. Their discovery in water, which was confirmed repeatedly thereafter, marked the beginning of discussion of their epidemiological importance, including the possibility of transformations into pathogenic agents through their adaptations to rodents.

Between 1926 and 1929 *L. grippotyphosa* was identified and a connection made with *Schlammfieber* (mud fever), described by F. Müller (1894). It was isolated from *Microtus avalis* by Van der Zaan (1941) and by Schüffner and Bohlander (1942). Klarenbeck and Schüffner identified *L. canicola* in dogs (1932); Clayton *et al.* (1937) did the same for *L. pomona* in Australia, Mino (1937) for *L. hyos* in Argentina, and Schüffner (1938) for *L. bataviae*. The number of leptospires identified will soon know no bounds.

This plurality was the result of largely successful attempts to uncover the aetiologies of fevers of an unknown origin (FUO). In Japan, investigators succeeded in attributing autumn fever and seven-day fever to *L. autumnalis* (Akiyama) and *L. hebdomadis*; in Australia, the connection was made between swineherd's disease and *L. pomona*; and in Switzerland Gsell (1946) unravelled the mystery of dairy flu and the pseudo-typho-meningitis of young swineherds.

This explosion of leptospires was identified on the basis of antigenic features of a rather heterogeneous kind. It made it necessary to keep large libraries of leptospiral identification plates, which is not feasible outside of specialized centres.

Field investigations, which began in Zaire in 1932, were therefore limited in terms of their geographical scope.

The possibility of leptospirosis in Zaire was raised for the first time by *Kadaner* and *Corti* (1933), following a small epidemic in 1932 which had affected fifteen Europeans swimming regularly in the Stanleyville (Kisangani) municipal pool. The aetiology was confirmed by sero-agglutination tests for three of the patients. The situation was repeated when a small epidemic broke out among the natives (Schwetz and Kadaner, 1934).

In 1936, Herman informed *J. Van Riel* of some cases of lymphocytic serous meningitis in workers of MGL (*Minière des Grands Lacs*) recruited outside the mining area and hospitalized at Kamituga, in the Zalya river basin. Spontaneous recovery was the rule. However, this infection spread little by little to workers of various origins. The clinical picture became clear, taking on the aspect of infectious hepato-nephritis with meningeal reactions. Of 32 cases diagnosed by Van Riel (1939), and by Van den Berghe and Van Riel (1939) from January 1937 through August 1938, three were fatal. The seroreactions performed at the Antwerp Institute of Tropical Medicine confirmed the diagnoses. The presence of leptospirosis was established by observations of leptospiruria and by the isolation of a leptospiral strain in August 1938.

This marked the start of exhaustive research carried out from 1939 to 1946 by J. Van Riel (1939 to 1955) and by J. and M. Van Riel (1956) in this Central African focus of leptospirosis. The dominant murine species, *Mastomys* (*Mastomys coucha* Smith), the house rat, was not a reservoir, whereas a strain of *Leptospira* (*L. ndambari* of the *icterohaemorrhagiae* serogroup) was isolated from *Arvicanthis abyssinicus*, a peridomestic rat.

Sero-epidemiological investigations aimed at detecting the disease reservoir yielded the following percentages of positive seroreactions: 13% in dogs (31:245); 5% in pigs (13:283); 34% in cattle (42:124); 3% in goats (11:353); and 4% in sheep (3:72).

Numerous serotypes were identified, without species specificity. The partial list includes *L. icterohaemorrhagiae* s.l., *L. hebdomadis, L. bataviae, L. grippotyphosa, L. butembo, L. australis* A, and *L. pomona*.

Human infection was studied in depth in 45 cases. The breakdown by five serotypes covered *L. grippotyphosa* 47%, *L. hebdomadis* 22%, *L. icterohaemorrhagiae* 12%, *L. bataviae* 11%, and *L. kisuba* 7%. No correlations could be established between the serotype and the severity of the illness.

The breakdown according to sex was 80 % for males (36 out of 45) and 20 % females (9 out of 45). This is the rule unless women's exposure is greater due to their occupations (female workers in fish industries or in rice fields).

Between 1936 and 1945, 364 cases of leptospirosis were observed in a focus near Lake Kivu.

Among them 18 (5%) were confirmed by isolation of the causal leptospire, 12 by blood cultures, 2 by detection in the CSF, and 4 by inoculation of guinea pigs with urine (Van Riel, 1946). In addition, 29 cases (8%) were identified by agglutination-lysis. One strain of *L. bataviae* was isolated from water on an alluvial mining operation.

Serological investigation of 1,077 serum specimens between 1946 and 1956 led to the identification of 42 strains belonging to 8 serogroups and included 11 new serotypes.

Van Riel et al. (1955) reported five cases of leptospirosis in Europeans living in the Belgian Congo. Two of the cases were caused by different leptospires of the *icterohaemorrhagiae* serogroup, one by a serotype of the *hebdomadis* serogroup, and the last two by *L. grippotyphosa.*

MAJOR CHALLENGES

1. Causal agent

The characteristic morphology of leptospires does not enable differentiation into species. The species are therefore identified on the basis of their antigens. The lipo-polysaccharide somatic antigens are specific to each species. The surface antigens, which are protein polysaccharides, are used to classify them into a large number of serovarieties (serotypes or serovars). However, these antigens are not stable. This heterogeneity compounded with frequent serotype associations can be very confusing.

The genus *Leptospira* has been divided into two species. The first assembles all of the proven or potential pathogens, with *Leptospira interrogans* as their representative. The second includes the non-pathogenic forms – water saprophytes – with *Leptospira biflexa* as their leader. *L. interrogans*, unlike *L. biflexa*, does not grow at 13°C and is sensitive to 8-azaguanine, but the two have some antigens in common.

As new strains are isolated and antigen analysis is refined, the number of serotypes has risen, increasing the need for taxonomic reorganization. There are so many serotypes that, for obvious practical reasons, efforts are focusing on reclassifying them into serogroups on the basis of their major antigens (Kmety). In 1948, Van Thiel mentioned 24 serotypes; this figure rose by 1954 to 34 (including 18 originating from Indonesia), classified into 20 serogroups (Wolff and Broom). Today, *L. interrogans* includes some 180 serovars, classified into 19 serogroups bearing some well-known names, as for example *icterohaemorrhagiae*, *canicola*, *grippotyphosa*, *pomona*, *autumnalis*, etc. In addition, the boundary between the two complexes is not absolute, the pathogenic *andamana* and *semaranga* serotypes, for example, being closer to *L. biflexa.*

Leptospires are relatively easy to culture, provided that some essential nutritional requirements (albumin, fatty acids, nitrogen) and environmental conditions (temperature, pH) are fulfilled. They grow slowly. The normal incubation time is two weeks, but can reach eight weeks for the *hardjo* serovar.

There is a wide range of recommended culture media, reflecting merely the subjective choices of leptospirologists. In practice, the semi-solid EM (Ellinghausen-McCullough) or Fletcher's medium will cover the usual requirements.

Young guinea pigs (120 to 140 g) and hamsters (18 to 25 g) can be inoculated intraperitoneally, but the animals must be observed daily. Mice and rats may be undetected carriers of leptospires. The aquatic leptospires can be isolated by using millipore filters.

2. Sources of contamination

The discovery by *Uhlenhuth* and *Zuelzer* (1921) of leptospires in water which are morphologically very similar to *L. icterohaemorrhagiae,* but have different antigenic and pathogenic features, continues to pose the problem of their possible role in the epidemiological chain. It has been proven in the laboratory that such apparently harmless, aquatic leptospires can be transformed into virulent strains in the guinea pig, the sewer rat (Zuelzer, 1931), and the white mouse (Thiry and Tiellin, 1932). Although this discovery can be important in basic research, in practice the role of this transformation is negligible.

The primordial role is played by water contaminated by leptospire-loaded urine. Infected water can be stagnant waters, sluggish water courses, swamps, marshes, mud, sludge, floodwaters, sewers, irrigation systems, water that has been used to wash stables and slaughterhouses, water accumulated in burrows and nests, polluted food, etc.

To survive in the environment leptospires require suitable pH and temperatures (around 25°C). Salinity and pollution are inhibiting factors.

The logical consequence is that their prevalence is not uniform, but varies over time and especially according to seasonal changes. Moreover, the soil type can be critical for survival. The presence of some heavy metals, copper in particular, can render the environment inhospitable.

The animal reservoir of leptospires is constituted by chronic mammalian carriers. Rodents – also the first animal reservoirs identified – make up a natural reservoir. They harbour the leptospires in a symbiotic relationship and excrete them in their urine to contaminate the water, the soil, etc., where the bacteria can survive for three or more weeks.

The dog came into the picture in 1932 as a facultative reservoir whose role is not negligible, although canine leptospiruria appears during the waning phase of a visible or unapparent pathogenic episode. Among the domestic animals, cattle, swine, and the Equidae are relatively important. Chronic infraclinical placental infections in these animals cause the deaths of young animals, spontaneous abortions, and mastitis, resulting in lower milk production and substantial economic losses, generally upsetting the international market.

A surprising variety of wild animals – birds, fish, frogs, *Viveridae* (civets, mongooses), carnivores, insectivores, bats, reptiles, and water turtles – help to maintain and spread leptospires in nature. However man's role is insignificant.

These reservoir animals are not specific for a given serogroup or serotype. Nevertheless, some more frequent associations do occur, such as the association between *L. pomona* and cattle; *L. canicola* and dogs; *L. icterohaemorrhagiae* and rats.

3. Transmission

The only mode of transmission is contact with leptospire-infected urine. Human contamination by direct contact is negligible. Licking, scratches, and bites have been cited, although leptospires have never been detected in the saliva. In animals, sexual and transplacental transmission has been shown to occur. The arguments in favour of a closed chain of transmission involving haematophagous arthropods (ticks) or nematodes are not very convincing. Person-to-person transmission is unlikely.

The pathogen is harboured by a secondary, essentially aquatic, medium. Water in all its forms – standing water, sluggish water, silts – plays a primordial role. Foodstuffs also could be a source of transmission, given their frequent pollution.

The leptospire enters the human body through the conjunctival, oro-naso-pharyngeal or digestive mucosa; and through skin which has been abraded or macerated by prolonged exposure in water.

The human host

Sero-epidemiological surveys show that symptomatic infections are not rare. Moreover, the majority of diagnosed clinical cases are self-limiting.

As a result, the effects of the presence of leptospires in infected humans are not known, especially since the pathogens leave no specific lesion at the point of entry. The site of multiplication is unknown. By analogy with observations of animal infections, it may be hypothesized that the leptospires persist and reproduce in the kidney's convoluted tubules, which they reach via the bloodstream. Based on data from clinical studies, the disease is dominated by specific tropisms: for the liver and for the meninges; for the kidney, with invasion and lesions of the tubular epithelium and renal dysfunction (see the chapter Renal diseases, p. 1163).

Virulence varies greatly, even for the same serotype. *L. Grippotyphosa* rarely causes serious illness in Europe, although it does in Israel. Some leptospires have a more pronounced tropism for specific organs such as *L. grippotyphosa*, *L. canicola*, and *L. pomona* which tend to invade the meninges and *L. icterohaemorrhagiae* invading the liver and the kidneys.

The host-pathogen relationship, governing the intensity of the reaction, depends on the leptospires' surface antigens. Tissue damage results from the lysis of the leptospires rather than from their presence. This should be remembered when leptospire-lysing antibiotics are used; however their therapeutic value is controversial.

It is also unnecessary to suppose that the leptospires have an endotoxin in order to interpret the basic lesion of leptospirosis which is the vascular involvement in various target organs, with lowered renal perfusion, haemorrhages, cholestasis, etc.

4. Epidemiology

This zoonosis, characterized by water-borne transmission and an extraordinarily rich diversity of animal reservoirs, is cosmopolitan. It occurs in tropical Africa and rages in Zaire. The conditions underlying the biological balance between leptospires and the aquatic environment in its broadest sense have not been sufficiently elucidated. The influence of the soil's geology

on leptospiral survival deserves serious study, as leptospires are found in over 80% of the collections of water located in sandy clays, whereas in crystalline gneiss the rate does not exceed 5%. *Sardjito* and *Zuelzer* (1928), working in Indonesia, noted that leptospires were present in the alkaline waters of Sumatra and absent in the more acid waters of Java, a fact that was confirmed by observations in the Andaman Islands, where leptospires are absent when the pH of water is below 6.6 (Taylor and Goyle, 1931).

The prevalence of leptospires in animals is not well known. The alkaline urine of pigs and herbivores, the natural animal reservoirs affecting man, is a very favourable medium and may contain up to 10,000,000 leptospires/ml.

The distribution of the serogroups and serotypes over the world is very uneven. In some countries the common serogroups, such as *pomona* and *grippotyphosa*, do not occur. Furthermore, it is not always enough to diagnose the group. In a given area, the serogroup *hebdomadis* can coexist in livestock and small rodents. The latter are suspected of constituting a reservoir for livestock. Actually, the *hardjo* serotype is more and more frequently isolated from cattle.

The seasonal pattern of the number of clinical cases is well known. Peaks occur in summer and autumn, a fact which is reflected in the common names for diseases identified as leptospirosis, such as autumn fever (Japan), vegetable growers or young swineherds' disease (Switzerland). This explains the increased risks of various occupations and activities causing direct or indirect contact with water possibly contaminated by the urine of reservoir animals. This is especially the case in rural environments.

The occupational categories requiring surveillance in tropical Africa include the farmers of sugar-cane and flooded rice, market gardeners, pig or cattle raisers, hunters, fish breeders, rubber tappers, agronomists, and veterinarians. Surprisingly, rat catchers or exterminators, who frequently come into contact with infected rats, are usually seronegative. The same goes for slaughterers. But workers in alluvial mining operations and workers at earth dams, navvies, and garbage collectors should be watched carefully. Stray dogs also constitute a risk.

As regards leisure activities, the protection of swimming pools and their water supplies from rats is of the utmost importance. Swimming, camping, water sports, occasional bathing and fishing could lead to suspicion of the disease. Schoolchildren should be watched. Contacts with pets or farm animals may give rise to family outbreaks. Leptospirosis may be suspected in individuals who have fallen accidentally into lakes or streams; it should also come to mind in the event of floods. More extensive data on the prevalence of leptospires in man and animals are needed. This is possible only through closer collaboration among physicians, veterinarians, epidemiologists, bacteriologists, and biologists.

5. The disease

The practitioner's ability to diagnose leptospirosis depends more on his epidemiological clearsightedness and critical mind than on classic descriptions of the disease as provided in old textbooks on Weil's disease or syndrome concerning relapsing jaundice. While this clinical form definitely does exist, it should not be accepted as the only or the dominant syndrome.

The clinical manifestations are more diverse than the classic picture and they range widely in severity. Most cases of contamination are subclinical or take the form of an influenza-like syndrome which disappears spontaneously. In addition, the incubation period can vary from three days to three months, complicating the history. One should pay attention to dilatation of blood vessels in the conjunctiva of the eyes without simple or purulent conjunctivitis. Serious cases often follow a biphasic course in which the invasion phase is followed by a one to three day silent period corresponding to an increase in circulating IgM: then there is a recrudescence marked by variable signs, such as catarrhal icterus (10%), meningeal involvement of variable intensity (40%), or the full syndrome (35%) of liver, kidneys, and meninges involvement with sometimes an extremely serious haemorrhagic syndrome. There is the risk of iridocyclitis as a late complication (appearing after four to eight months) due to the persistence of leptospires or immune complexes in the anterior chamber of the eye. Hospital practice in Zaire shows a lymphocytic meningitis as the main involvement.

For the first strains isolated from patients with fevers of unknown origin, connections were found between autumn fever and *L. icterohaemorrhagiae*, between swineherd's disease and *L. pomona* and between mud fever and *L. grippotyphosa*. Nevertheless no correlation was found between the serogroups or serotypes and the clinical picture, nor any specificity for the reservoir animals.

Clinical suspicion based on a suggestive history will become a certainty when leptospires are isolated and identified. This is sometimes possible by direct observation of urine against a dark background from day seven (D7) onwards. The usual practice will be to

culture blood collected on liquid during acute episodes (at D1 to D8) or CSF collected at D3 to D10. Liquid of the anterior chamber of the eye also contains leptospires. Culture of urine is possible over a longer period, despite a high failure rate. Contaminants, often present in urine, can be eliminated by neomycin or cycloheximide. These methods require a specialized laboratory. The same applies when laboratory animals are inoculated, although intraperitoneal injections may be performed on site.

In practice, diagnosis relies on serotests. Their interpretation is based on the significant increase of antibody levels taken at convalescence compared to those of the acute phase.

The available techniques have a wide range. First macro-agglutination tests are applied using a battery of reference antigens chosen for the area and the specific serogroups. Then more precise tests will be conducted. Nevertheless, the sharing of antigenic characteristics, even major ones, and the frequency of simultaneous or repeated infections, lead to scattered serovars. Beside this a large number of co-agglutinins and the trailing by related serotypes increase the serological confusion. Paradoxical reactions, such as higher titres for non-specific antigens than for homologues, complicate the situation further during fairly extended periods.

The tests of choice are haemagglutination which detects circulating IgM antibody as of day D4, complement deviation, micro-agglutination, ELISA, radioimmunoassay (RIA), and indirect fluorescent antibody (IFA) technique. Agglutination-lysis is the most specific test, combined if necessary with antigen absorption, but this technique requires live cultures and a vast leptospire serovar library. Differential diagnosis covers aseptic meningitis, viral hepatitis, and various viral and bacterial infections.

6. Treatment and prevention

Various antibiotics – penicillin, tetracyclines, erythromycin – kill leptospires *in vitro*. However, their usefulness *in vivo* is doubtful. If some reports are favourable they are not backed up by valid controls. Moreover, leptospirosis usually disappears on its own. Finally, to administer a lysing medication is risky during the leptospiraemic phase and useless during the immune phase.

Serious cases will get more benefit from supportive treatment given for an hepato-nephritic or haemorrhagic syndrome than from specific treatment.

The measures recommended by some authors to control the animal reservoirs (rat extermination, separating man's living quarters from those of domestic animals) are illusory. It is useful to monitor swimming pools and their water supply systems to prevent contamination by rodents. Vaccinating infected livestock helps only the carrier, as it does not halt excretion of the leptospires in the urine. Immunizing people at risk, while technically feasible, is not justified, due to the leptospires' extreme antigenic variability.

7. Problem for further study

While this zoonosis is not a public health priority, it can still be a potential problem for some groups of people, such as alluvial mineworkers, navvies, and especially workers in agro-pastoral industries. Herdsman and shepherds of small flocks can act as indicators of an infectious risk liable to cause serious economic losses for stock-farming.

Since the sixties, leptospirosis has been neglected in Zaire and neighbouring countries. Active screening aimed at high-risk groups would be desirable.

From a scientific point of view such studies would make it possible to harvest a large number of known or as yet unknown serovars. The mass of information to be gleaned would enable reclassification of the various groups and a better selection of the battery of reference antigens. Up to 1950, diagnoses and prevalence rates were established only on *L. icterohaemorrhagiae* and *L. canicola* antigens.

P.G. Janssens and J. Van Riel (†)

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This dissertation is the result of meticulous research in Kenya on acute leptospiroses in man. This work includes an important bibliography covering the whole of Africa.

INSTITUTE OF TROPICAL MEDICINE PRINCE LEOPOLD (1966) International Colloquium on Leptospirosis, (3-5 déc. 1965), – *Ann.Soc. Belg. Méd.Trop.*, 46, pp. 1-270.

Organized to celebrate the fiftieth anniversary of the discovery of *Spirochaeta icterohaemorrhagiae*, this colloquium assembled the world's leading leptospirologists and contains a number of extremely valuable communications.

The statements of L.H. Turner and the remarks made by C. Borg-Petersen make fundamental contributions to leptospiral taxonomy.

The separation and characterization of the serotypes, even sub-serotypes, based on the variability of the leptospires' antigenic structure are an occasion for interesting discussions. Research conducted by E. Kmety and co-workers on the major antigens constituted a basis for further thinking. Epidemiological data are provided for Europe.

KABAMBA – KAJIMA S.G. (1971), *Les Leptospiroses en Afrique Noire. A propos d'une enquête microbiologique et sérologique menée au Sénégal,* Thèse de doctorat, n° 15, Faculté mixte de médecine et de pharmacie, Université de Dakar, Sénégal.

This excellent doctoral thesis contains invaluable information on the problem of leptospirosis and a study of its importance in Senegal, but also valuable specific data on leptospirosis in Kenya and Zaire.

RYU E. (1978-1980) *Chronological References of Zoonoses: Leptospirosis and Leptospiroses*, 2 vol., 359 p.

A chronological review of all contributions published between 1812 and 1977. An author and subject index make research easy.

VAN RIEL J. (1946), Le foyer centro-africain de leptospirose (Contribution au problème de l'unité ou de la pluralité des leptospires du type L. *icterohaemorrhagiae*), – *Ann. Soc. Belg. Méd. Trop.*, 26, pp. 197-313.

While taking account of the first observations of leptospirosis in Stanleyville (Kisangani), the author's personal investigations in the mining area of Kivu constitute the first deeply grounded observations of leptospirosis in the Central African focus and the plurality of the agents (*L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. bataviae*). The diversity of clinical manifestations resulting from the triple tropism for the liver, kidneys, and meninges is illustrated by case studies with pre-eminence of meningeal irritation.

The role of rodents as reservoirs of the disease and their diversity are studied, with special attention given to the role of *Arvicanthus abyssinica*. The epidemiology of the disease, including the possible role of leptospires living in water, is the occasion for a highly instructive discussion of the problem of the unity or plurality of leptospires.

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The new leptospirosis focus studied in this dissertation was observed in the cassiterite (tin) mines of Kabunga, in Eastern Zaire. After describing the clinical picture for most of the 45 cases studied, the authors present in detail the results of the laboratory diagnostic methods used. They then study the epidemiology, from which they conclude that water plays the main role. At least five leptospire serotypes were observed in this focus. This contribution includes an extensive list of references.