Leptospirosis: a zoonotic disease of global importance

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In the past decade, leptospirosis has emerged as a globally important infectious disease. It occurs in urban environments of industrialised and developing countries, as well as in rural regions worldwide. Mortality remains significant, related both to delays in diagnosis due to lack of infrastructure and adequate clinical suspicion, and to other poorly understood reasons that may include inherent pathogenicity of some leptospiral strains or genetically determined host immunopathological responses. Pulmonary haemorrhage is recognised increasingly as a major, often lethal, manifestation of leptospirosis, the pathogenesis of which remains unclear. The completion of the genome sequence of *Leptospira interrogans* serovar *lai*, and other continuing leptospiral genome sequencing projects, promise to guide future work on the disease. Mainstays of treatment are still tetracyclines and β-lactam/cephalosporins. No vaccine is available. Prevention is largely dependent on sanitation measures that may be difficult to implement, especially in developing countries.

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Leptospirosis is a zoonotic disease of global importance.[1] In recent years, endemic and epidemic severe pulmonary haemorrhage has increasingly become recognised as an important manifestation of leptospiral infection.[2–5] Leptospirosis has also emerged as a disease of the adventure traveller, especially affecting participants in water-sports.[6,7] It has a worldwide distribution but is more common in the tropics where conditions for its transmission are particularly favourable. However, disease continues to occur in developed countries,[6] for example among holiday-makers in Hawaii[8] or sporadically in inner-city residents.[9] Important advances have been made in diverse aspects of this emerging infectious disease. Although leptospirosis does not have the potential to be used as a weapon, its clinical manifestations can mimic those of viral haemorrhagic fevers, meriting attention in the age of bioterrorism.

Microbiology and taxonomy

Leptospires are spirochetes (figure 1), a group of bacteria that diverged early in bacterial evolution.[10] The family leptospiraceae includes two genera, *Leptospira* and *Leptonema*. Typically, leptospires were classified according to antigenic determinants.[11,12] More recently, a molecular classification has been described that divides the *Leptospira* genus into several species on the basis of DNA relatedness.[13–16] The reclassification of leptospires using genetic determinants provides useful taxonomic information, but is independent of the established serological classification with which epidemiologists and clinicians are more familiar. Hence, serovar and serogroup designations will continue to be used for the foreseeable future.

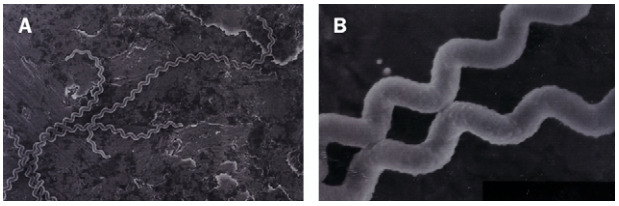

Figure 1. High-resolution scanning electron micrograph of Leptospira interrogans *serovar* copenhageni*. (A) Note characteristic hooked ends. (B) At high magnification the surface of the spirochete seems ruffled and beaded. The leptospires were grown in vitro, fixed in cacodylate buffer, dehydrated through ethanol, processed through hexamethyldisilazine, air dried, and visualised without metal coating (x3000). Courtesy of Vsevolod Popov and Violet Han, Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA.*

Microbiology

The leptospiral genome consists of two circular chromosomes[17] and its entire sequence was recently established.[18] The genome is large compared with the genomes of other spirochetes such as *Treponema* spp[19] and *Borrelia* spp,[20] which indicates the ability of *Leptospira* spp to live within diverse environments: animal hosts and freely in the environment. Little is known about genetic exchange among the *Leptospira*, although lateral transfer has been suggested.[21] Tools for genetic manipulation of leptospires are being developed for studies of pathogenesis, virulence

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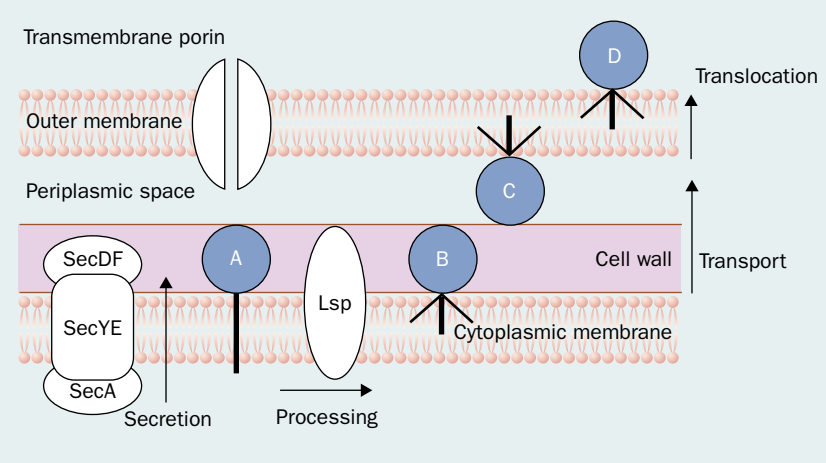

Figure 2. Schematic depiction of the structure of leptospira. A=prolipoprotein; B=subsurface lipoprotein in the cytoplasmic (inner) cell membrane; C=subsurface lipoprotein in the inner leaflet of the outer membrane; D=surface-exposed lipoprotein (possible antigenic determinant) in the outer leaflet of the outer membrane; Lsp=prolipoprotein signal peptidase. Modified from reference 23.

Table 1. Classification of *Leptospira* species

Species	Serovar	Reference strain	Serogroup
Pathogens			
L interrogans	*australis*	Ballico	Australis
	bratislava	Jez Bratislava	Australis
	bataviae	Van Tienen	Bataviae
	canicola	Hond Utrecht IV	Canicola
	hebdomadis	Hebdomadis	Hebdomadis
	icterohaemorrhagiae	RGA	Icterohaemorrhagiae
	copenhageni	M 20	Icterohaemorrhagiae
	lai	Lai	Icterohaemorrhagiae
	pomona	Pomona	Pomona
	pyrogenes	Salinem	Pyrogenes
	hardjo	Hardjoprajitno	Sejroe
L alexanderi	*manhao3*	L 60	Manhao
L fainei	*hurstbridge*	BUT 6	Hurstbridge
L inadai	*lyme*	10	Lyme
L kirschneri	*bim*	1051	Autumnalis
	cynopteri	3522 C	Cynopteri
	grippotyphosa	Moskva V	Grippotyphosa
	mozdok	5621	Pomona
	panama	CZ 214K	Panama
L meyeri	*semaranga*	Veldrat Semaranga 173	Semaranga
L borgpetersenii	*ballum*	Mus 127	Ballum
	castellonis	Castellon 3	Ballum
	javanica	Veldrat Bataviae 46	Javanica
	sejroe	M 84	Sejroe
	tarassovi	Perepilitsin	Tarassovi
L weillii	*celledoni*	Celledoni	Celledoni
L noguchii	*fortbragg*	Fort Bragg	Autumnalis
L santarosai	*brasiliensis*	An 776	Bataviae
	georgia	LT 117	Mini
Genomospecies 1	*pingchang*	80-412	Ranarum
Genomospecies 4	*hualin*	LT 11-33	Icterohaemorrhagiae
Genomospecies 5	*saopaulo*	Sao Paulo	Semaranga
Saprophytes			
Genomospecies 3	*holland*	Waz Holland (P438)	Holland
L biflexa	*patoc*	Patoc I	Semaranga
L wolbachii	*codice*	CDC	

Adapted from Levett PN. Leptospira and leptonema. In: Murray PR, Baron EJ, Pfaller MA, et al, eds. Manual of clinical microbiology, 8th edn. Washington DC: ASM Press, 2003: 929–36.

factors, and basic cell biological studies of the organism.[22]

Leptospires are highly motile, obligate aerobic spirochetes that share features of both Gram-positive and Gram-negative bacteria (figure 2).[23] Leptospires are about 0·25 × 6–25 μm in size and can pass through 0·45 μM filters. Dark-field or phase-contrast microscopy of wet preparations is required for direct visualisation of leptospires, since the bacteria stain poorly. Electron microscopy shows a cylindrical cell body (protoplasmic cylinder) wound helically around an axistyle (0·01–0·02 μm in diameter), which comprises two axial filaments (a spirochetal form of a modified flagellum) inserted subterminally at the extremities of the cell body, with their free ends directed towards the middle of the cell (figure 1).[24] An external sheath envelops the axistyle and protoplasmic cylinder, which is demarcated by a cytoplasmic membrane.[25] The axial filament is thought to be a cytoskeletal element that enables movement.[25] It is attached to the inner surface of the membrane and periodically contracts, causing rotation of the spiral and thus movement.[25]

The appearance and motility of leptospires varies with the nature of the medium in which they are grown. In liquid media, cells appear bent or hooked at one or both ends, although straight mutants do exist. In some cultures, leptospires may appear as small granules (1·5–2·0 μm in diameter) containing coiled remnants of the leptospiral cell. Three types of movement are possible: rotation around a central axis, progressive movement in the direction of the straight end, and circular motion. In semisolid media, motion is by means of flexion. Newly isolated leptospires appear shorter on initial subculture with even higher translational and helical motility.[26]

Leptospires are cultivated in artificial media containing 10% rabbit serum[27] or 1% bovine serum albumin plus long-chain fatty acids at pH 6·8–7·4. Optimum growth temperature is between 28°C and 30°C. Leptospires are catalase and oxidase positive. Cultures should be checked

Table 2. Serogroups of *Leptospira interrogans* sensu lato of clinical importance with some associated serovars

Serogroup	Serovar(s)
Australis	*australis, bratislava*
Autumnalis	*autumnalis, fortbragg, bim*
Ballum	*ballum, arborea*
Bataviae	*bataviae*
Canicola	*canicola, portlandvere*
Celledoni	*celledoni*
Cynopteri	*cynopteri*
Djasiman	*djasiman*
Grippotyphosa	*grippotyphosa*
Hurstbridge	*hurstbridge*
Hebdomadis	*jules*
Icterohaemorrhagiae	*icterohaemorrhagiae, copenhageni, lai*
Javanica	*javanica*
Louisiana	*lanka*
Lyme	*lyme*
Manhao	*manhao*
Mini	*georgia*
Panama	*panama*
Pomona	*pomona*
Pyrogenes	*pyrogenes*
Sejroe	*sejroe, hardjo*
Tarassovi	*tarassovi*

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for the presence of contaminating bacteria after 3–4 days and subcultured after 7–21 days, although leptospires can survive in undisturbed liquid culture for months, sometimes years.[28] Media are made selective by the addition of several antibiotics, the most common being 5-fluorouracil and neomycin sulphate, although polymyxin B, rifampicin, and vancomycin have been used.[27] A commonly used medium is Ellinghausen-McCullough-Johnson-Harris (EMJH) medium,[29–31] which contains 1% bovine serum albumin and Tween 80 (source of long-chain fatty acids); commercial formulations are available. Serum-containing liquid or semisolid media include Korthof's (peptone, NaCl, $NaHCO_3$, KCl, $CaCl_2$, KH_2PO_4, Na_2HPO_4) and Fletcher's (peptone, beef extract, NaCl, and agar).[28]

Taxonomy

The species classification of the genus *Leptospira* is based on DNA relatedness (table 1).[13–16] The genus is divided into 17 species, defined as being at least 70% DNA-related and whose related DNA sequences contain at most 5% unpaired

Table 3. Typical reservoir hosts of common leptospiral serovars

Reservoir host	Serovar(s)
Pigs	*pomona, tarassovi*
Cattle	*hardjo, pomona*
Horses	*bratislava*
Dogs	*canicola*
Sheep	*hardjo*
Racoon	*grippotyphosa*
Rats	*icterohaemorrhagiae, copenhageni*
Mice	*ballum, arborea, bim*
Marsupials	*grippotyphosa*
Bats	*cynopteri, wolffi*

Table 4. Leptospiral serovars seen in multiple species

Serovar	Species
bataviae	*L interrogans, L santarosai*
bulgarica	*L interrogans, L kischneri*
grippotyphosa	*L interrogans, L kischneri*
hardjo	*L borgpetersenii, L interrogans, L meyeri*
icterohaemorrhagiae	*L interrogans, L inadai*
kremastos	*L interrogans, L santarosai*
mwogolo	*L interrogans, L kischneri*
paidjan	*L interrogans, L kischneri*
pomona	*L interrogans, L noguchii*
pyrogenes	*L interrogans, L santarosai*
szwajizak	*L interrogans, L santarosai*
valbuzzi	*L interrogans, L kischneri*

Adapted from Levett PN. Leptospira and leptonema. In: Murray PR, Baron EJ, Pfaller MA, et al, eds. Manual of clinical microbiology, 8th edn. Washington DC: ASM Press, 2003: 929–36.

bases (divergence).[15] This classification coexists with the older serological classification in which antisera are used to establish antigenic relatedness between isolates.[11] Leptospiral strains are still commonly referred to by serovar (tables 1 and 2). Many serovars studied are represented by only a single reference strain, and as more strains are studied the number of species is likely to increase.[32]

Some leptospiral serovars are commonly associated with particular animal reservoirs (table 3). Typically, leptospires were divided into two serological species, with most known or suspected pathogenic leptospires grouped within the "interrogans" complex (later, *Leptospira interrogans* sensu lato). All others were placed in the "biflexa" complex (later, *Leptospira biflexa* sensu lato), which contained primarily the saprophytic strains. Both complexes (*L interrogans* and *L biflexa*) have been divided into several serovars using the cross-agglutinin adsorption test (CAAT).[11,12] Antigenically related serovars are arranged for convenience into serogroups. More than 60 serovars of *L biflexa* sensu lato have been described and more than 200 serovars, arranged into 24 serogroups, are recognised within *L interrogans* sensu lato.[32] Both the antigenic and the more recently developed genetic classification systems of *Leptospira* are in use because genetic characterisation is possible in only a few research laboratories and reference serological reagents (polyclonal and monoclonal antibodies) capable of defining serovars are not readily available. Further, neither serovars nor serogroups are indicative of the taxonomic relation among strains, because one serovar (defined by antibodies directed against its lipopolysaccharide antigen) may belong to more than one species (table 4) and members of the same genetic group do not necessarily belong to the same serogroup.[33] Consequently, new *Leptospira* isolates should be characterised by both molecular and serological approaches.

Epidemiology

Leptospirosis has a worldwide distribution. The incidence of human infection is higher in the tropics than in temperate regions but transmission occurs in both industrialised and developing countries. Incidence rates are underestimated due to lack of awareness of the disease and relatively inaccessible and insufficiently rapid diagnostics. Symptom-

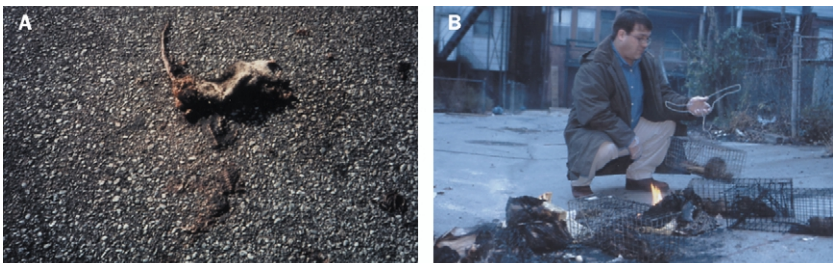

Figure 3. Urban mammalian reservoirs of leptospiral transmission. (A) A dead rat in a Baltimore, MD, USA, alley where almost all rats were leptospiruric and were the source of infection for sporadic human cases, as reported in reference 9. (B) While trapping rats in another Baltimore alley, the senior author encountered an expression of local residents' feelings about sanitation in their neighbourhood—one of the local citizens placed trapped rats on a bonfire and burned them alive.

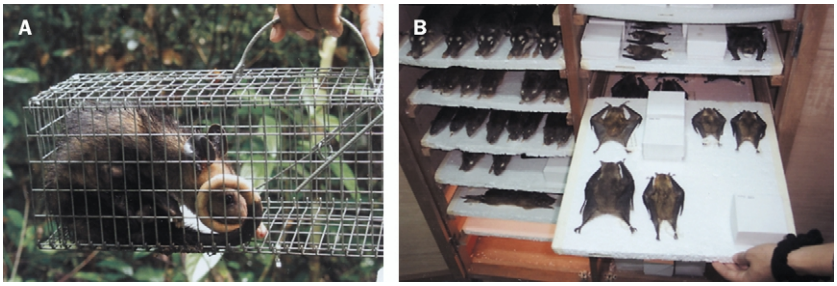

*Figure 4. Neotropical mammalian reservoirs of leptospiral transmission. (A) A neotropical opossum (*Didelphis marsupialis*) trapped in the Peruvian Amazon near Iquitos. Culture of this animal's kidney yielded a pathogenic leptospiral isolate with a novel molecular fingerprint as determined by pulse field gel electrophoresis. (B) A collection of neotropical rodents, marsupials, and bats, in which pathogenic leptospires are commonly seen. See reference 47.*

less or subclinical infection is common in endemic regions.[34,35] We have seen that a surprisingly high proportion (20–30%) of patients presenting with acute undifferentiated fever to local health posts in the Iquitos area of Peru have serological evidence of acute leptospirosis.

Leptospirosis is maintained by the persistent colonisation of the proximal renal tubules of carrier animals. An infected animal can remain symptom-free and shed infectious organisms in the urine for its entire lifetime.[28,36,37] Human beings have never been proven to be important epidemiological sources of transmission, although individuals can excrete leptospires into the urine for weeks or, more rarely, months or more than 1 year (J M Vinetz, unpublished findings).[28] Human infection results from exposure to infected urine of carrier mammals, either directly or via contamination of soil or water. The prevalence of different leptospiral serovars within a human population depends on the reservoir animals present and the serovars that they carry, as well as local environmental conditions, occupation, agronomical, and agricultural practices. Several host–serovar associations seem to be ubiquitous—for example *Rattus* species and serovar *icterohaemorrhagiae*, and mice and serogroup Ballum serovars (table 3). Studies have shown that isolated populations of mammals may be important in the maintenance of unusual serovars, such as the carriage of serovar *bim* by house mice (*Mus musculus*) in Barbados.[38] Moreover, a single species may carry different serovars in geographically distinct populations, as exemplified by the small Indian mongoose (*Herpestes auropunctatus*), which maintains serovars *sejroe* and *icterohaemorrhagiae* in Hawaii,[39] serovars *icterohaemorrhagiae* and *djatzi* in Puerto Rico,[40] serovars *icterohaemorrhagiae* and *jules* in Jamaica,[41] serovars *icterohaemorrhagiae* and *brasiliensis* in Grenada,[42] and serovar *canicola* in Trinidad.[43]

Leptospirosis was formerly considered to be primarily an occupational disease, associated with activities such as mining, sewer maintenance, livestock farming and butchering, veterinary medicine, and military manoeuvres. The relative importance of such occupational risks has decreased since protective measures have been implemented. In developed countries, many cases occur in association with conditions of slum living (figure 3)[9,44] or with recreational activities involving immersion in water.[6,7,45] In tropical environments, occupational exposure such as rice farming and other agricultural activities is still significant, but so too is exposure of the general population during activities of daily living, and especially is associated with high seasonal rainfall (figures 4, 5, and 6).[2,46–48] Of significance is the potential for large, multinational, point-source outbreaks after recreational events.[49]

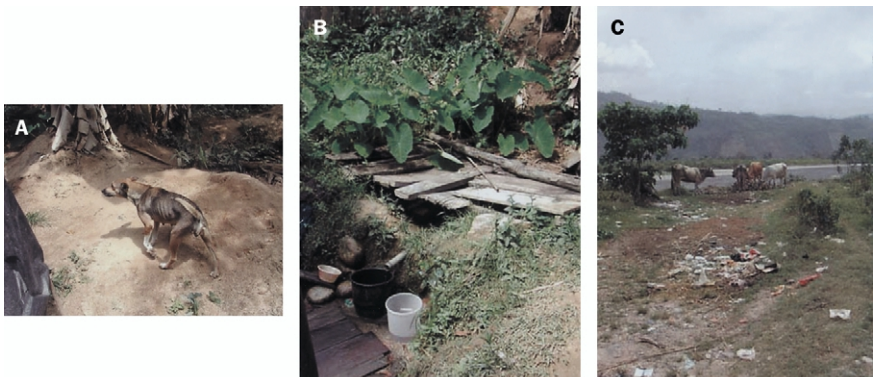

Figure 5. Domestic animals as potential reservoirs of leptospiral transmission. These scenes are from the high jungle in the interior of Peru, near Pichanaqui, in the Chanchamayo Valley (where yellow fever is endemic), waters from which feed into the Amazon basin. An outbreak of leptospirosis affecting a group of military recruits occurred here.[48] (A) A typical domestic dog, poorly cared for, that lives outside. (B) A well from which water is taken for domestic use, and into which the dog in (A) just urinated. (C) Cattle adjacent to the Pichanaqui River, where the soldiers in the outbreak swam, and near a rubbish heap where rats are seen.

The biodiversity of leptospires in the environment is affected by geography, climate, biotic inter-

Figure 6. Common ecological and epidemiological contexts of leptospirosis transmission in Peru. (A) Walking barefoot through a deforested area near the Amazon city of Iquitos. (B) A fresh water swimming hole with swimmers barely visible in the background, near the village of Santa Clara just outside Iquitos. (C) A well serving as a source of potable water for domestic use. (D) Women washing clothes in the village of Varillal, near Iquitos.

actions, and anthropogenic activities (figures 3, 4, and 5).[9,47,48] Environmental conditions strongly affect the transmission of leptospirosis by modifying the population biology, behaviour, or community ecology of spirochetes and their hosts. Leptospiral diversity is limited on islands such as Barbados, where only four pathogenic serovars infectious to people have been identified,[38,50] and in urban environments where the major potential reservoir mammals are limited to rats and dogs.[9,44] In tropical regions with high species richness, such as the Amazon basin or other continental settings like rural southeast Asia, wild mammals would probably be infected by leptospires, and these leptospires should be highly diverse.[51,52] Indeed, we have confirmed this to be the case. A wide range of neotropical mammals including rodents, bats, and marsupials (figure 4),[47] were shown by a specific PCR assay to have a high prevalence of leptospiral renal carriage.[47] In a continuing study of the ecology of leptospirosis transmission in the Peruvian Amazon, we have additionally obtained several leptospiral isolates from both wild and domestic animals, including known leptospiral serovars such as *icterohaemorrhagiae*, as well as what seem to be four novel leptospiral isolates based on pulsed field gel electrophoresis patterns. These isolates were obtained from the kidneys of domestic rats (*Rattus norvegicus* and *Rattus rattus*, one of which had the same molecular type as that isolated from a person), a spiny rat (*Proechimys* sp), an opossum (*Didelphis marsupialis*), and two other marsupials, four-eyed opossums (*Philander andersoni* and *Philander opossum*) from three different habitats (forest, secondary

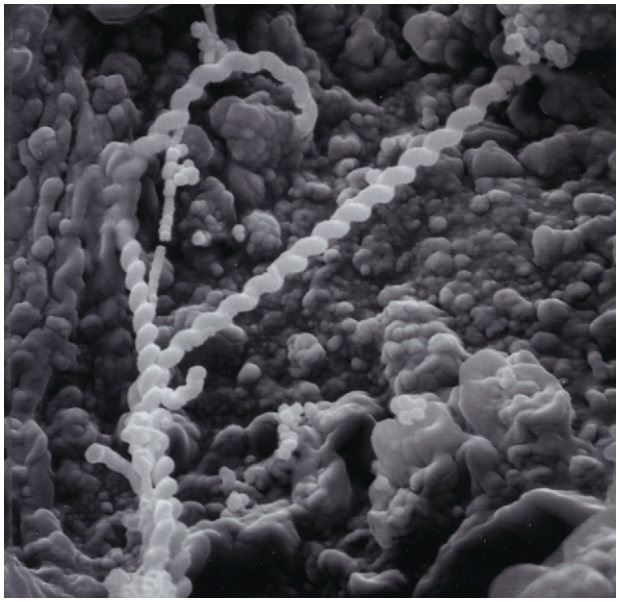

Figure 7. Scanning electron micrograph of adhesion/invasion of a pathogenic leptospire to equine conjunctival epithelium.

growth, and cultivation areas; M M Diaz, C H Estrada, and M R Willig, unpublished findings).

Pathogenesis

Host infection by pathogenic *Leptospira* produces a diverse array of clinical manifestations ranging from subclinical infection to undifferentiated febrile illness to jaundice, renal failure, and potentially lethal pulmonary haemorrhage. Our understanding of mechanisms of leptospirosis pathogenesis is limited. Answers to the most basic questions, such as whether the outcome of infection (mild or severe disease) is due to direct pathogen effects or genetically determined host immune responses, remain elusive. The highly variable clinical manifestations of leptospiral infection suggest that a diverse range of events may contribute to acute and chronic infection processes of people and reservoir hosts. This supposition is supported by the recently released complete genome of the pathogenic *L interrogans* serovar *lai*, which comprises 4768 predicted genes, more than four times the number predicted for other sequenced spirochetes.[18–20]

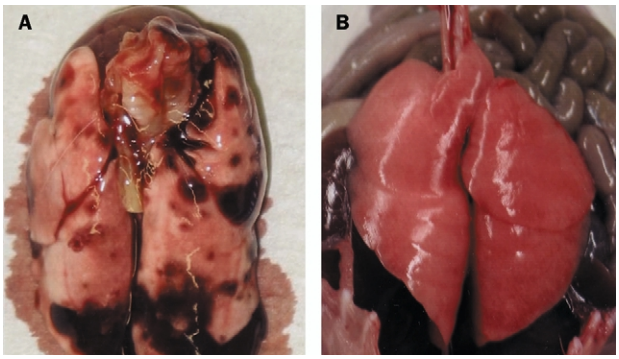

Figure 8. (A) Pulmonary haemorrhage in a guineapig infected with a strain of L interrogans *serovar* copenhageni *obtained from a Brazilian patient with pulmonary haemorrhage. (B) Lungs from a normal guineapig are shown at right for comparison.*

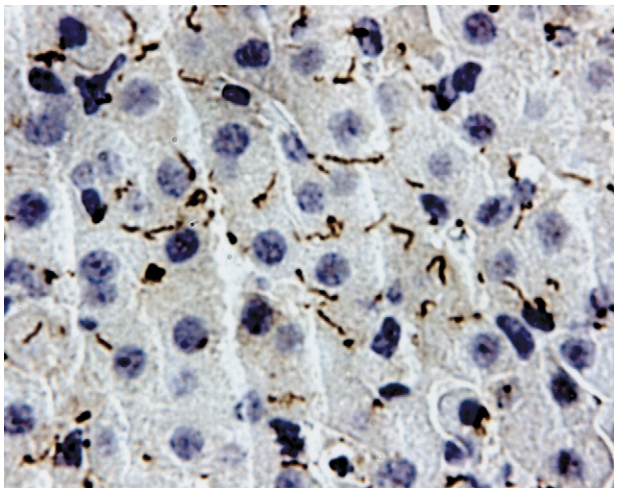

Figure 9. Immunohistochemistry showing leptospires (stained brown) in liver from a guineapig infected with a strain of L interrogans *serovar* copenhageni *obtained from a Brazilian patient with pulmonary haemorrhage.*

Pathogenic mechanisms of leptospirosis may be divided into direct effects by *Leptospira* and host immune response to infection. One mechanism of virulence is motility and the ability of *Leptospira* to swim through viscous media.[28] Motility is probably important in initial infection and in dissemination of organisms from the site of entry to sites of end-organ damage such as lung, liver, kidney, eye, and brain. Of the 4768 predicted genes identified in the genome sequence, at least 50 are related to motility.[18] Associated with motility, 12 methyl-accepting chemotaxis proteins, which are likely to confer selective advantages in adapting to and migrating through host tissues, were also identified.[18,53] Virulent *Leptospira* strains, but not avirulent or saprophytic strains, have been shown to exhibit chemotaxis towards haemoglobin.[54] Consistent with the predicted ability to migrate through host tissues, *Leptospira* have a range of

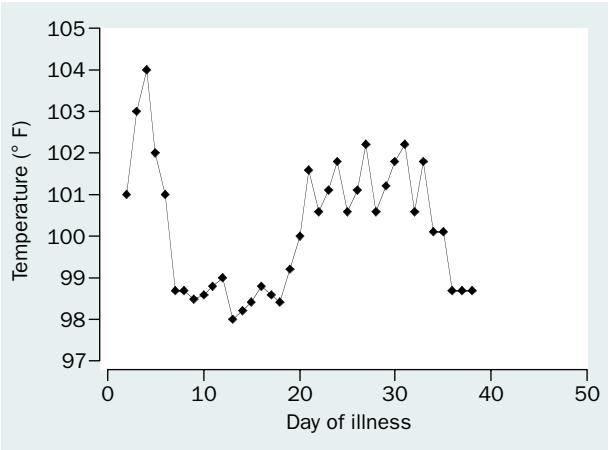

Figure 10. Course of leptospirosis in a patient with a biphasic illness, characterised by an undifferentiated febrile syndrome accompanied by pyuria in the first phase, in whom fever recurred in the context of aseptic meningitis in the second phase. Microscopic agglutination testing showed a titre of 1/6400 with L interrogans *serovar* ballum*. The patient was a graduate student who had been responsible for cleaning his own mouse cages in a poorly constructed animal housing facility, in which cages were not protected with microisolator tops.*

potential virulence factors that may facilitate this process. Haemolytic, sphingomyelinase, and phospholipase activities have been described in vitro[55] and, subsequently, specific genes have been characterised including haemolysins, sphingomyelinase C, sphingomyelinase H, and haemolysis-associated protein-1 (Hap1, also known as LipL32).[56–58] By contrast with sphingomyelinase C, sphingomyelinase H showed no sphingomyelinase activity but was shown to be a cytotoxic pore-forming protein on several mammalian cells.[58] Several additional haemolysins and sphingomyelinase-like proteins have been identified in the genome sequence of serovar *lai*. The in-vivo relevance of these potential virulence factors in the pathogenesis of leptospirosis has not been established.

A fibronectin-binding protein specifically expressed on the surface of virulent *L interrogans* serovar *icterohaemorrhagiae*, but not on avirulent strains, may be significant in initial adhesion and invasion at cutaneous or mucosal sites of entry (figure 7).[59] Since *Leptospira* regulate expression of proteins in response to environmental stimuli, particularly with differences in protein expression between in-vitro and in-vivo contexts,[60–62] a fundamental question for efficient diagnostic and vaccine development will be to address which genes are expressed during infection compared with in-vitro conditions. The leptospiral immunoglobulin-like protein A (LigA) contains domains homologous to proteins with attachment and invasion functions, and is expressed in vivo but not in vitro.[63] Four genes related to the

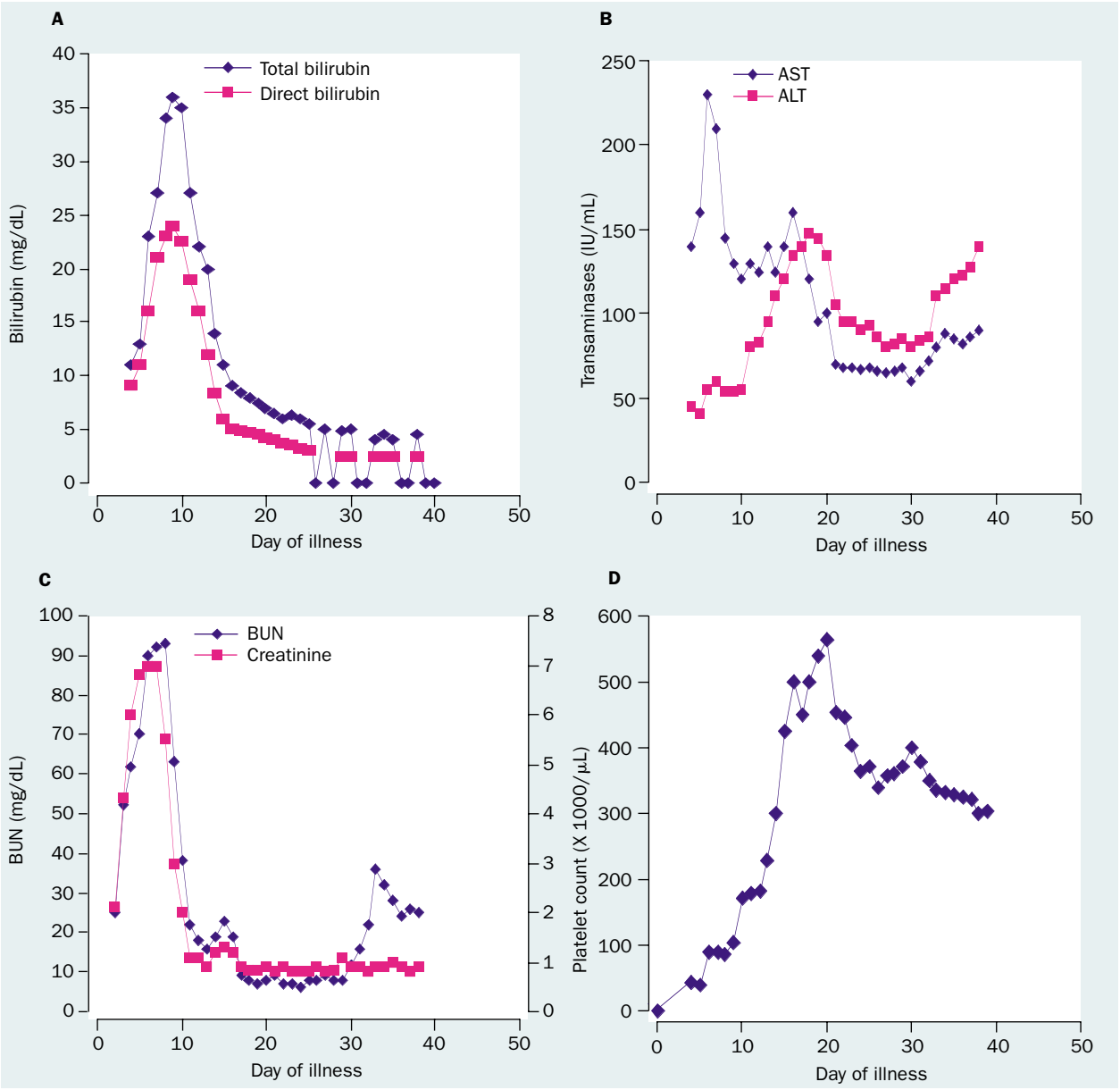

Figure 11. Course of biochemical and platelet abnormalities in a patient with severe leptospirosis manifested as Weil's disease (jaundice, renal failure, haemorrhage). (A) Bilirubin levels. (B) Transaminase levels. (C) Renal function. (D) Platelet count. The case was originally described in reference 9. AST=aspartate aminotransferase; ALT=alanine aminotransferase; BUN=blood urea nitrogen.

attachment and invasion of eukaryotic cells were identified in the serovar *lai* genome sequence including homologues of the mammalian cell entry gene *mce* of *Mycobacterium tuberculosis* and the invasion gene *invA* of *Rickettsia prowazeskii*.[18]

Infection of experimental animal models provides a range of clinical manifestations depending on the age and species of animal model, the virulence of the infecting strains, and the inoculated dose (figures 8 and 9). Chronically infected animals are usually symptom-free, harbouring *Leptospira* in the proximal renal tubules, which allows for dissemination to the environment via urine. A recent study of seemingly healthy dogs in Kansas, USA confirmed such an event. Of 500 dogs assessed without regard to health status, 41 were shown to have leptospiruria by PCR,[64] and only four had clinical findings consistent with leptospirosis. We recently saw that of about 1200 cattle serum specimens from a Texas slaughterhouse, 262 (22%) had leptospiral antibodies as established by microagglutination testing, with high titre antibodies (1/800 or greater) in several clusters of feed lots that send cattle to this slaughterhouse. A subset of 300 urine samples obtained from this cohort of cattle was tested for leptospiral DNA by a polymerase chain reaction assay; of these, 106 (35%) were positive, indicating excretion of leptospires.[65] These dog and cattle data from the USA are probably typical of many industrialised and developing countries, and suggest an ongoing threat of leptospirosis transmission to people in a range of settings.

Histological examination of kidneys of infected carriers may show interstitial nephritis believed to be a direct result of the presence of *Leptospira* in tissue, but chronic carriers typically have no renal pathology. Addition of an outer membrane protein preparation from a serovar *shermani* strain to mouse proximal renal tubule cells in vitro caused a dose-dependent production of chemokines (monocyte chemoattractant protein 1 [MCP]1, RANTES, nitrite, and tumour necrosis factor [TNF] α) which would probably contribute to inflammation.[66] More specifically, recombinant-LipL32-stimulated expression of MCP1 and inducible nitric oxide synthase mRNAs, and augmented nuclear binding of nuclear factor kappa B and AP1 transcription factors.[66] Lipopolysaccharide and the outer membrane protein OmpL1 are also implicated in interstitial nephritis.[67] Leptospiral lipopolysaccharide is considerably less toxic than is typical Gram-negative lipopolysaccharide, with different biochemical, physical, and biological properties.[28] These properties include activation of macrophages via Toll-like receptor (TLR) 2 instead of the more conventional TLR4 for typical Gram-negative lipopolysaccharide.[68]

Experimentally infected animals with acute infection indicate the more serious icteric Weil's disease reported in human patients and, in particular, the more serious haemorrhagic syndromes associated with leptospirosis (figure 8).[69] In acutely infected animal models, liver and kidney pathology seems to be related to large numbers of leptospires and associated cytotoxic factors in tissues (figure 7).[70,71] *L interrogans* glycolipoprotein inhibits sodium-potassium ATPase pump activity in a dose-dependent manner in rabbit renal tubule cells and activates peripheral blood mononuclear cells.[72,73] By contrast with liver and kidney, few *Leptospira* are seen in experimentally infected guineapig and hamster lung tissues with severe pulmonary haemorrhage.[74,75] Lung pathology, where much lower numbers of leptospires per g of lung tissue relative to liver and blood counts have been reported, may be due to exposure of circulating toxins produced at distant sites such as the liver.[76] However, the lower numbers of leptospires in haemorrhagic lung tissue also supports an indirect pathogenic mechanism mediated by the host immune response to infection. Patients with an IgG titre of 400 (n=13) or more had more severe pulmonary haemorrhage and renal function damage compared with patients with an IgG titre of less than 400 (n=22).[77] By contrast with studies on human patients,[78–80] severe haemorrhage in guineapigs is thought to be mediated by disseminated intravascular coagulation.[81,82] Interestingly, the serovar *lai* genome sequence suggests alternative pathogenic mechanisms

Table 5. Relative frequencies (percentage of people affected) of various clinical manifestations reported in case studies of confirmed dengue (type 1), Mayaro virus disease, Oropouche fever, Venezuelan equine encephalitis, psittacosis, leptospirosis, parvovirus B19, and Venezuelan haemorrhagic fever

Signs and symptoms	Leptospirosis	Mayaro virus	Oropouche fever	VEE	Psittacosis	Primary dengue	Parvovirus B19	VHF
Fever	97	100	97	100	100	98	27	93
Headache	98	100	88	89	87	96	28	58
Myalgia	79	74	82	66	75	93	NR	40
Eye pain	NR	63	NR	15	NR	91	14	NR
Arthralgia	23	47	67	11	NR	88	7	53
Chills	78	58	85	33	61	88	NR	NR
Rash	7	21	NR	1	NR	71	85	NR
Nausea/vomiting	41	21	26	39	49	62	7	35
Cough	20	16	3	NR	78	55	19	20
Diarrhoea	29	11	13	22	20	28	7	27
Sore throat	14	16	NR	20	17	NR	51	36
Number of cases reported in study	771	19	68	79	135	150	162	55

NR=not recorded; VEE=Venezuelan equine encephalitis; VHF=Venezuelan haemorrhagic fever. Adapted from Vinetz JM. 10 common questions about leptospirosis. *Infectious Diseases in Clinical Practice* 2000; **9:** 19–25.

Table 6. Signs and symptoms of leptospirosis in hospitalised patients in large case series

Symptoms	% of patients							
	China, 1995 (115) n=75	Puerto Rico, 1963 (18) n=208	China, 1965 (615) n=168	Vietnam, 1973 (61) n=150	Korea, 1987 (442) n=93	Barbados, 1990 (177) n=88	Seychelles, 1998 (660) n=75	Brazil, 1999 (332) n=193
Jaundice	72	49	0	1·5	16	95	27	93
Anorexia	92	NR	46	NR	80	85	NR	NR
Headache	88·5	91	90	98	70	76	80	75
Conjunctival suffusion	97	99	57	42	58	54	NR	28·5
Vomiting	51	69	18	33	32	50	40	NR
Myalgia	100	97	64	79	40	49	63	94
Arthralgia	51	NR	36	NR	NR	NR	31	NR
Abdominal pain	31	NR	26	28	40	43	41	NR
Nausea	56	75	29	41	46	37	NR	NR
Dehydration	NR	NR	NR	NR	NR	37	NR	NR
Cough	55	24	57	20	45	32	39	NR
Haemoptysis	37	9	51	NR	40	NR	13	20
Hepatomegaly	83	69	28	15	17	27	NR	NR
Lymphadenopathy	19	24	49	21	NR	21	NR	NR
Diarrhoea	30	27	20	29	36	14	11	NR
Rash	0	6	NR	7	NR	2	NR	NR

Adapted from reference 32. NR=not recorded.

because several proteins with homology to animal proteins important in haemostasis were identified, including platelet-activating factor acetylhydrolase and von Willebrand factor type A domains.[18] These virulence factors may directly activate haemostasis pathways or, alternatively, induce an autoimmune response. Autoimmunity is believed to be the underlying pathogenic mechanism in ocular leptospirosis, a chronic condition noted in people and horses.[83,84] Leptospira have been detected by molecular techniques in vitreous and aqueous humour from naturally occurring cases of equine recurrent uveitis.[84,85] Furthermore, a locally produced antibody response mediates fixation of the complement cascade component C3 to equine cornea, which shares antigenic epitopes with *L interrogans*.[86,87]

We still do not have detailed knowledge of mechanisms of host immunity to *Leptospira* or the role of host immunity in leptospirosis pathogenesis. Naturally acquired immunity that protects against reinfection by *Leptospira* does occur and has been assumed to be humourally mediated.[88–90] Protective immunity may be engendered by antibodies directed against serovar-specific leptospiral lipopolysaccharide. Leptospiral lipopolysaccharide stimulates the innate immune system via a TLR2–dependent mechanism, another potential mechanism of either protective immunity or immunopathogenesis.[68] Some evidence suggests that antibodies against *Leptospira* membrane-associated proteins may have a role in host defence, but such evidence is not definitive.[89,91] High-grade bacteraemia (10^1–10^6/mL) in acute leptospirosis may occur despite moderate or high titre leptospiral agglutinating antibodies,[92] suggesting that alternate mechanisms other than antilipopolysaccharide antibodies could have a role in naturally acquired protective immunity.

The role of cell-mediated immunity in leptospirosis is being explored. Studies of cattle given a killed *L borgpetersenii* vaccine have shown that immunised cattle have CD4+ T cells and γδ T cells that give in vitro proliferative responses and produce interfon γ after stimulation with a *Leptospira* antigen preparation.[93] TNFα and interleukin 10 seem to be upregulated by a leptospiral glycolipoprotein, which was reported to stimulate CD69 and HLA-DR expression on peripheral blood mononuclear cells (PBMCs).[72] Treatment of weanling C3H/HeJ mice with a CD4 monoclonal antibody exacerbated pathology during infection with a virulent strain of *L interrogans* serovar *icterohaemorrhagiae*.[94] Animal models and human clinical studies provided indirect evidence that TCRγδ+ T cells may play an important part in host defence against bacterial, viral, and parasitic infections in general. We have reported that human PBMCs from leptospirosis-naive individuals are stimulated to produce large quantities of interferon-γ-producing TCRγδ+ T cells during in-vitro stimulation by pathogenic leptospires,[95] and that PBMC-derived dendritic cells, when stimulated by leptospires, secrete interleukin 12 (G R Klimpel, M A Matthias, J M Vinetz, unpublished findings). Further, we have reported in a small number of patients presenting with an acute undifferentiated febrile illness who are dipstick positive for leptospirosis antibodies, that TCRγδ+ T-cell concentrations are increased in peripheral blood.[95] The in-vivo role of TCRγδ+ T cells in terms of their relation to pathogenesis, protection (or neither), or cell-mediated immunity in general, remains to be elucidated.

In summary, much remains to be established in the cellular and molecular mechanisms underlying the clinical expressions of leptospirosis. Leptospira are highly effective pathogens, as shown by their ubiquitous distribution and diversity of pathogenic mechanisms. The continued elucidation of pathogenic mechanisms in relevant animal models should lead to improved patient treatments, efficient diagnostic assays, and effective vaccines.

Clinical features

Leptospirosis has protean manifestations, and mimics the clinical presentations of many other diseases.[32,96] Consequently, mechanisms of the clinical manifestations of leptospirosis remain obscure. Typical descriptions[97] include a biphasic illness (anicteric form, figure 10) and fulminant disease (icterohaemorrhagic form, figure 11).[9] In the biphasic illness the initial acute or septicaemic phase is characterised by bacteraemia that typically lasts about 1 week. Most of the recognised cases present with a febrile illness of sudden onset. Multiple clinical reports have indicated that fever is present in most or all cases.[98] A substantial proportion of people infected by *Leptospira* may have subclinical disease or very mild symptoms, and do not seek medical attention. Symptomless infection is common and has been reported in several studies.[34,35] An investigation of a 1995 outbreak of leptospirosis in Nicaragua reported that only 25 (29·4%) of the 85 seropositive inhabitants reported a febrile illness in the 2 months before the survey.[35] In a study in the Seychelles, 9% of men had laboratory evidence of recent infection, and 37% had evidence of past infection, with no-one reporting current or past symptoms of leptospirosis.[34]

In our experience with a prospective cohort epidemiological study in the Peruvian Amazon city of Iquitos, we saw an estimated incidence rate of leptospiral seroconversion of 288/1000 in the urban slum of Belen. Seroconversion in this setting was associated with an antecedent history of fever in some but not all patients (M A S Johnson, R H Gilman, J M Vinetz, et al, unpublished findings). Collectively, these findings suggest that symptomless infection with *Leptospira* is common in endemic areas. It has not been shown in human beings whether pre-existing leptospiral antibody in symptom-free individuals may protect against severe leptospirosis in endemic areas but these patients are unlikely to have a role in transmission since person-to-person spread of this disease is rare.[99] Fever, chills, headache, severe myalgia, conjunctival suffusion, anorexia, nausea, vomiting, and prostration usually characterise acute leptospirosis (table 6). This finding was confirmed by a population-based study of leptospirosis in Hawaii, where fever, myalgia, and headache were the most frequently reported symptoms.[100] Nausea and vomiting were also common and jaundice was seen in 39% of cases. No significant association was seen between any particular leptospiral serovars and the clinical outcome of infection. Conjunctival suffusion and muscle tenderness, most notably in the calf and lumbar areas, have been mentioned as distinguishing physical findings.[32,97]

The resolution of symptoms may coincide with the immune phase when antibodies begin to be produced, accompanied by excretion of spirochetes in the urine. However, fever may recur after a remission of 3–4 days, producing a biphasic illness (figure 10). In most cases, the biphasic disease is not clinically distinguishable from other undifferentiated febrile illness syndromes (table 5). Headache is often severe, resembling that of dengue, with retro-orbital pain and photophobia, and may be associated with cerebrospinal fluid (CSF) pleocytosis ranging from 10–1000 white blood cells/μL with a polymorphonuclear predominance. The CSF may be culture or PCR positive.

Aseptic meningitis may be seen in up to one-quarter of all leptospirosis cases. The neurological manifestation of leptospirosis in the first phase is dominated by clouded sensorium and meningism followed by the second phase, which is characterised by typical neurological features that include headache, vomiting, and signs of meningeal irritation. Examination of the CSF shows increased opening pressure, raised protein, normal glucose, and lymphocytic pleocytosis. Although antibodies can be detected during this phase, *Leptospira* cannot be isolated. It is uncommon for leptospirosis to present as a primary neurological disease.[101]

Weil's disease represents only the most severe form of the illness. This syndrome can develop after the acute phase as the second phase of a biphasic illness, or simply present as a single, progressive illness. It is characterised by jaundice, renal failure, and haemorrhage with a variable clinical course (figure 11).[9] The case fatality rate may be high, ranging from 5–15%. The icteric form of the disease is seen in 5–10% of all patients with leptospirosis.[102] Serum bilirubin concentrations may be high (up to 30–40 mg/dL) and take days to weeks to normalise (figure 11).[9] Transaminase concentrations are typically moderate (in the 100s) with minor increase of alkaline phosphatase concentrations (figure 11).[9] The jaundice in leptospirosis does not seem to be due to hepatocellular damage, but seems to be more related to the cholestasis of sepsis,[103] with impairment of the ATP-dependent secretion of conjugated bilirubin into the bile canaliculi. Platelet counts may be very low and contribute in part to the haemorrhagic diathesis (figure 11D). Liver function returns to normal with recovery from illness without sequelae.

Acute renal failure is reported in 16–40% of cases,[104] and is usually non-oliguric. Oliguria is a significant predictor of death (OR 9·0).[105] Serum amylase rates are often increased in patients with acute renal failure but clinical symptoms of pancreatitis are not common.[106] Indeed, leptospirosis may mimic pancreatitis or cholecystitis (fever, right upper quadrant pain, Murphy's sign); leptospires are seen in the surgically extirpated gall bladder wall. Thrombocytopenia is typical (figure 11D), develops in up to 50% of patients with leptospirosis, correlates with the occurrence of renal failure, and is associated with a poorer prognosis.[107] Thrombocytopenia in human beings does not seem to result from a pathophysiological process of disseminated intravascular coagulation.[78]

The true incidence of pulmonary involvement is unclear but may range from 20–70%.[3] Patients may present with symptoms ranging from cough, dyspnoea, and haemoptysis, to adult respiratory distress syndrome (table 6). The severity of respiratory disease is unrelated to the presence of jaundice.[108] Radiography generally shows a patchy alveolar infiltrate that may coalesce to form larger areas of consolidation,[109] which indicate areas of intra-alveolar and interstitial haemorrhage. Pleural effusions may occur. Alveolar infiltrates and dyspnoea are poor prognostic indicators in severe leptospirosis.[110] In patients with pulmonary involvement, haemodynamic disturbance (OR 6·0), serum creatine concentration above 265 μmol/L (OR 10·6), and serum potassium concentration above 4·0 mmol/L (OR 19·9) were associated with mortality.[111]

Cardiac involvement is probably more common than is reported. In mild disease, electrocardiogram abnormalities may be non-specific.[112] In a series of patients from the Philippines with severe leptospirosis, electrocardiogram abnormalities were seen in 13 (48%) of 27 patients. First degree atrioventricular block and changes suggestive of acute pericarditis were the most frequent abnormal findings. The P-R interval normalised in all patients on follow-up. Other abnormalities included T-wave inversions, S-T segment increases, and dysrhythmias. There was no association between cardiac involvement and skeletal muscle injury.

Nearly all patients with acute leptospirosis experience severe myalgia, and most show evidence of mild rhabdomyolysis.[113] Severe rhabdomyolysis has been reported but is rare.[114] Creatine kinase increase in a jaundiced patient with a mild to moderate increase in serum transaminases should raise the consideration of leptospirosis, as opposed to viral hepatitis. Mechanisms that induce rhabdomyolysis remain to be elucidated. Speculation has included consideration of spirochetal release of a toxin that damages muscle directly and the possibility that leptospires invade muscles leading to inflammation and destruction.[115]

Ocular manifestations have been long recognised in leptospirosis and, as shown in a series of cases of ocular leptospirosis occurring after flooding in India,[83] have several manifestations.[116] Conjunctival suffusion and muscle tenderness are important distinguishing physical findings.[6,97] A large cluster of cases of sight-threatening uveitis after an outbreak of leptospirosis were reported from India.[83] Anterior uveitis occurs after recovery from acute illness in a few cases.[117] Uveitis is an important late complication that can cause reversible or irreversible blindness in people and in horses.[116] It is presumed to be an immune event, but leptospires have been shown in aqueous humour by PCR.[118] Subconjunctival haemorrhage, chorioretinitis, papilloedema, papillitis, optic neuritis, retinal bleed, and cotton-wool spots are other manifestations of leptospirosis in the eyes.[116]

The differential diagnosis of leptospirosis must take into account diseases that are locally prevalent, and which can present as undifferentiated fever, such as malaria, rickettsioses, arboviral infections (dengue, yellow fever, and others), etc. Diagnosis must also include common viral infections such as influenza, HIV seroconversion, and, in the presence of pulmonary involvement, hantavirus infection.

Severe febrile illness with haemorrhagic manifestations may make leptospirosis clinically indistinguishable from viral haemorrhagic fevers.[119,120] Scrub typhus is reportedly similar to leptospirosis clinically and there may even be coinfection with the two organisms, as seen in Thai agriculture workers.[121]

Diagnosis

General laboratory tests

Diagnosis of leptospirosis depends on simple diagnostic tests, which are often not done because of a low index of clinical suspicion. Laboratory diagnosis of leptospirosis can be made either by showing the organism or by serological tests that detect leptospiral antibodies.[122] Several non-specific findings may include increased ESR, mild increases in transaminases, alkaline phosphatase, and bilirubin; abnormal urinanalysis showing proteinuria, pyuria, and microscopic haematuria. CSF protein may be normal or slightly raised, and CSF glucose is usually normal. In patients with severe jaundice, xanthochromia may occur. Initial CSF examination may show predominance of polymorphs or lymphocytes that is followed by lymphocyte preponderance. In severe leptospirosis, there is peripheral leucocytosis with a left shift. The platelets are decreased[107] and renal function is impaired, as indicated by raised plasma creatinine concentrations. In icteric patients, the increase in bilirubin is generally out of proportion to other test values of liver function.[107] Serum creatine phosphokinase and serum amylase also may be raised. Such findings by routine laboratory tests only suggest a diagnosis of leptospirosis; specific microbiologic tests are needed for confirmation. Dark-field microscopy to see organisms in blood or urine is fraught with false-positives and false-negatives, and is not recommended.[1]

Culture

The definitive diagnostic test is the recovery of leptospires from clinical specimens, either by culture, which is insensitive and slow, by immunohistochemical staining, or by showing the presence of leptospiral DNA by PCR. Procedures to culture pathogenic *Leptospira* have changed little in recent years. Leptospires can be isolated from blood and CSF samples during the first 7–10 days of illness, and from urine during the 2nd and 3rd week of illness.[122–124] Culture is difficult, requires several weeks of incubation, and has low sensitivity; the specialised culture media are stocked in few clinical laboratories. Blood and CSF specimens can be collected in heparin or sodium oxalate for transport at room temperature; citrate anticoagulation should be avoided since it is inhibitory,[125] and specimens should not be frozen. Media should be inoculated within 24 h. If routine blood cultures are set up, leptospiral cultures can be established by subculture of the primary culture into leptospiral culture medium.[126,127] Even under optimum conditions, organisms grow slowly and cultures can be reported as negative only after a minimum of 6–8 weeks, preferably as long as 4 months.[122,124]

Molecular methods

One surrogate for direct demonstration of leptospires in human samples is diagnosis based on PCR. A real-time quantitative PCR assay using TaqMan chemistry to detect leptospires in clinical and environmental samples has been reported.[128] This PCR assay is sensitive and can differentiate between pathogenic and non-pathogenic species, although further studies need to confirm this, and has important implications for patient care because the diagnosis can be made early. Moreover, this method can be used in patients who have been started on antibiotics already. We have tested several standard clinical blood-collection systems to look at the interference of chemical components with the PCR sensitivity. Only those collection systems that contained lithium heparin interfered with the PCR sensitivity. In addition, quantification could suggest the burden of disease and may be prognostically useful.[92]

Serology

Serology is the most frequently used diagnostic approach for leptospirosis. The microscopic agglutination test (MAT) is the reference standard test for serological diagnosis of leptospires because of its high sensitivity and specificity.[129,130] The MAT detects agglutinating antibodies in serum, but requires significant expertise from its users, and interlaboratory variation in results is high. The standard criterion for a positive MAT are a fourfold increase in antibody titre, or a conversion from seronegativity to a titre of 1/100 or above. The results of this assay have been used to infer the identity of the infecting leptospiral serovar or serogroup. In a large case series that reviewed culture-positive cases in Barbados over 18 years, it was not possible to predict the infecting serogroup in more than half the cases.[122] Because the range of serovars and serogroups in Barbados is narrow and well-defined, it is likely that the sensitivity and specificity of MAT is higher in this setting than might be seen elsewhere. Serological study of patient serum samples does not seem to be helpful in identifying the infecting serovar in individual cases, but may be useful in predicting serogroups present within a population.[122]

Rapid genus-specific tests have been used widely for diagnosis. These tests have the advantage of providing rapid results without the need for culture or MAT facilities. Many tests have been described,[131] but those in contemporary use are primarily IgM-detection assays. IgM antibodies against leptospires become detectable during the first week of illness,[131–134] when specific antibiotic treatment is most likely to be effective. Most assays use crude whole-cell lysates as antigens, but recently recombinant cell-surface lipoprotein antigens have been assessed.[135] Several assays are commercially available.[131,136–139] The chief advantage of such assays is that they often become positive before the MAT.[129]

Treatment

There remains some controversy about whether antimicrobial treatment of severe leptospirosis should even be initiated since most cases of acute leptospirosis resolve spontaneously.[140] However, most experts would not withhold antimicrobial treatment when clinical findings and epidemiological exposure history suggest leptospirosis.[140] In the case series reported from Hawaii from 1974–1998, no significant difference was seen between use and non-use of antibiotics and duration of illness.[100] In a more recent prospective, randomised controlled trial of 79 patients (38 treated with penicillin G and 41 untreated patients), there were no differences with respect to time required for normalisation of biochemical parameters, duration of fever, or mortality.[141] However, several case series have reported shortened duration of illness when appropriate antibiotic therapy was administered during the initial phase of the illness (within 2–4 days). In patients with severe disease, late administration of antibiotics has also shown clinical efficacy and reduction in mortality rates.[134] A Cochrane database review that assessed antibiotic effectiveness in leptospirosis concluded that, because of a small number of published randomised clinical trials, there was insufficient evidence to provide clear guidelines for practice.[142] However, suggestive evidence supported the use of penicillin and doxycycline.[142] Treatment of leptospirosis patients continues to be supportive management and use of appropriate antibiotics. Currently recommended regimens and dosages are based on the severity of the disease. Doxycycline is recommended for both prophylaxis and mild disease.[98,143] Ampicillin and amoxicillin are also recommended in mild disease, whereas penicillin G and ampicillin are indicated for severe disease.[134]

In an important recent advance, Panaphut and colleagues[144] compared the efficacy of ceftriaxone and penicillin for the treatment of acute severe leptospirosis. Patients with high likelihood of severe leptospirosis based on clinical presentation of fever with jaundice, increased serum creatinine, and/or hypotension were selected. Initial laboratory diagnosis was based on a commercially produced dipstick assay that detected genus-specific leptospiral IgM antibodies. Of 372 clinically suspected cases, 173 had positive dipstick assays and 162 completed the trial. Of the 173 dipstick-positive patients, 110 had definitive evidence of leptospiral infection as indicated by MAT (100 with a fourfold increase in titre and ten with seroconversion). Over a follow-up period of about 1 week, the median duration of fever in both groups was 3 days. There were no significant differences in complications between the two groups and the mortality rates were identical (overall case fatality rate of 5·8%). Ceftriaxone has the benefit of reduced frequency (once a day versus every 4 hours for parenterally administered penicillin) and the option of intravenous and intramuscular administration. It is also more cost-effective than penicillin, and in patients with penicillin allergy it may be an alternative antibiotic.

The susceptibility of *L interrogans* serovar *icterohaemorrhagiae* strain Verdun to selected antibiotics used in medical practice (ampicillin, doxycycline, and ofloxacin) was assessed in a Syrian hamster model.[145] A quantitative PCR assay was used to monitor the density of leptospires in the blood and in target organs (liver, kidney, lung, heart, and spleen). Doxycycline (10 mg/kg) cleared the leptospires from blood and all tissues in 2 days, except for liver, which required 3 days. Ampicillin (100 mg/kg) cleared leptospires from the host, except for kidneys and heart, which still had 10^2 leptospires/g at day 6. Ofloxacin (30 mg/kg) was unable to clear bacteria from blood or kidneys. It is difficult to show conclusively that quantitative PCR data indicate the presence of viable leptospires in target organs, and the clinical relevance of this finding is unknown.

Leptospires are sensitive in vitro to most antimicrobial agents, but the relevance of the in-vitro findings to clinical outcome for these agents has not been assessed in clinical trials. A recent report[146] shows that while *Leptospira* are sensitive in vitro to several antimicrobial classes, some variability was reported in the in-vitro susceptibility of various *Leptospira* species to a range of newer (ampicillin-sulbactam, cefotaxime, ceftriaxone, azithromycin, telithromycin, ciprofloxacin, moxifloxacin) and old antimicrobials (penicillin, ampicillin, amoxicillin, doxycycline, tetracycline, chloramphenicol, erythromycin). Many of the *Leptospira* species tested were more sensitive to ampicillin/sublactam than to ampicillin alone.

Chemoprophylaxis

In a now classic study, a clinical trial comparing doxycycline (200 mg/week) with placebo was done in Panama in 1982 involving 940 US soldiers deployed for jungle training.[98] 22 cases of leptospirosis occurred in the placebo group (attack rate of 4·2%), which was significantly different from the single case in the doxycycline group (attack rate of 0·2%, p<0·001). A randomised clinical trial done on the North Andaman islands of India examined doxycycline prophylaxis against leptospirosis in inhabitants of a highly endemic area.[147] A sample population of 782 people was split into two randomised groups, and was given doxycycline (200 mg/week) or placebo. MAT was done on blood samples obtained at day zero, 6 weeks, and 12 weeks. No difference was seen in infection rate between the two groups as shown by seroconversion, but a significant difference was present in the clinical disease attack rate (3·1 *vs* 6·8 %). The results suggest that doxycycline prophylaxis does not prevent leptospiral infection in an endemic area, but may have a significant protective effect in reducing morbidity and mortality, even in an endemic setting. Chemoprophylaxis may be impractical to administer in highly endemic areas, but is likely to be useful for adventure travellers and military personnel who visit endemic areas, and also in accidental laboratory infection. Assessing the utility and practicality of antileptospiral prophylaxis after severe events such as floods and hurricanes would be a valuable clinical study.[46,83,148]

Vaccine development

Vaccines to prevent human leptospirosis are available in some countries and large-scale clinical trials have been reported from Cuba,[149,150] Russia,[151] and China[152] in non-English language journals. In Cuba, there was not a single reported side-effect in more than 100 000 people vaccinated and protection was reported to be 100%.[149,150] Only a few patients developed MAT antibodies to the serovars in the preparation,[149,150] so that in-vitro tests did not correlate with protective immunity. Long-term efficacy studies of antileptospiral vaccines have not been published, and it is likely that killed bacterial vaccines have only short-term efficacy, necessitating repeated vaccination to maintain immunity, with the attendant side-effect profiles that might be expected.

Several problems confront the development of a vaccine to prevent human leptospirosis. First, an unacceptable side-effect profile of killed bacterial vaccines has often been reported. Second, the killed bacteria vaccines are likely to provide only short-term and possibly incomplete protection, similar to that reported with antileptospiral vaccines in animals. Third, the locally varying patterns of *Leptospira* transmitted may preclude the development of a suitably generalisable vaccine. Fourth, there is theoretical potential for inducing autoimmune disease such as uveitis[83,153–155] and, lastly, there is incomplete knowledge of mechanisms of protective immunity against leptospiral infection. Vaccination of animals such as dogs or cattle may prevent illness but not leptospiruria and hence transmission to human beings.[156,157]

Substantial evidence from animal models indicates that lipopolysaccharide antibodies against homologous *Leptospira* mediate protective immunity.[28,32] Whether the same is true in people has not been shown. There is some evidence to suggest the possibility that cellular mechanisms of immunity (both innate and acquired) may also be involved in protective immunity, in in-vivo cattle[93] and mouse models,[94] and in vitro.[68,95] Serum samples from people with leptospirosis contain antibodies that recognise several protein antigens from the outer membrane, periplasmic space and the outer membrane, as well as serovar-specific lipopolysaccharide.[158] The current emphasis in research laboratories is to discover cross-species-conserved or cross-serovar-conserved protective antigens[89,91] that may provide longer-term protection from a broad range of *Leptospira*. Probably the greatest barrier to antileptospiral vaccine development is the practicality of developing a polyvalent leptospirosis vaccine for human beings in endemic areas who may be exposed to several serovars.

Search strategy and selection criteria

We identified relevant English language publications from 1966 onwards through Pubmed searches. Keywords used were "leptospirosis" and "leptospira". To look for reports of human vaccine clinical trials in all languages we used keywords "vaccine", "leptospirosis", and "clinical trials". We also examined reference lists of major reviews, a reference database compiled by Solomon Faine, and the compendium of references by E Ryu, Chronological references of zoonoses: leptospires and leptospirosis, 2nd edn. National Taiwan University: Taipei, 1979. We also drew on our own field and laboratory experiences to augment the review.

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Conflicts of interest

We have no conflicts of interest.

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