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Primer

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Leptospirosis

Senaka Rajapakse[1] ✉, Narmada Fernando[2], Anou Dreyfus[3], Chris Smith[4,5] & Chaturaka Rodrigo[6]

Abstract

Leptospirosis is a zoonotic bacterial infection that is prevalent across all continents and is caused by pathogenic spirochaetes of the genus *Leptospira*. Although infection can be asymptomatic, symptomatic disease can vary in severity from mild to severe illness, the latter characterized by icterus and/or multi-organ dysfunction and potentially death. An estimated one million cases of leptospirosis occur globally each year, resulting in ~60,000 deaths. The pathogenesis of severe leptospirosis is poorly understood but is believed to involve an interplay between genetic predisposition, pathogen virulence and dysregulated immune responses that trigger a cytokine storm with associated immunoparesis. *Leptospira* are susceptible to several low-cost antibiotics, including benzyl penicillin, doxycycline, cephalosporins and macrolides, when used in the early phase of infection. Late disease with organ dysfunction is treated with supportive care, and the benefit of antibiotics during late disease is doubtful. Very few countries have licensed a vaccine for human leptospirosis, and available vaccines only protect against rodent-associated serogroups. Exposure control by behavioural modifications and personal protective measures are the major preventative measures in leptospirosis, and the efficacy of prophylactic antibiotics has not been confirmed in clinical trials. Future research is needed to accurately estimate leptospirosis disease burden across the globe, to understand the pathophysiology of severe leptospirosis to inform the design of targeted immunotherapies and vaccines, and to develop cost-effective and accurate point-of-care diagnostics.

Sections

[1]Department of Clinical Medicine, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka. [2]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Colombo, Sri Lanka. [3]Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland. [4]Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK. [5]School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan. [6]Department of Pathology, School of Biomedical Sciences, Faculty of Medicine and Health, University of New South Wales, Sydney, New South Wales, Australia. ✉e-mail: senaka@med.cmb.ac.lk

Introduction

Leptospirosis is a globally prevalent zoonotic disease that is caused by free-living, Gram-negative, aerobic, flagellated *Leptospira* (phylum Spirochaetota). Natural reservoirs for the infection include over 160 species of mammals, which spread the pathogen through their body fluids (such as urine), contaminating waterways and soil[1]. Humans, who are incidental hosts, become infected by direct contact with infected animals or by exposure to contaminated water or soil, through a breach in the skin or penetration of mucous membranes or the conjunctiva[2] (Fig. 1). Human-to-human transmission is unconfirmed, with anecdotal evidence that is limited to a few case reports[3,4].

Leptospires are highly motile flagellated bacteria with characteristic hooked ends, approximately 6–20 µm in length and 0.1 µm in diameter[5]. They have a double membrane structure (outer and inner membranes) that surround an inner peptidoglycan layer[6]. Outer membrane proteins (OMPs) and their associated lipopolysaccharides (LPS) have an important role in disease pathogenesis and severity. Early taxonomic classification divided the *Leptospira* genus into pathogenic (*L. interrogans*) and saprophytic (*L. biflexa*) species. Each species was further subdivided into serovars based on surface antigen expression[7], and serovars with similar combinations of surface antigens were assigned to serogroups. As whole *Leptospira* spp. genomes were sequenced, its phylogenomic taxonomy has been updated to include 68 species of *Leptospira*[8], which are divided into two clades (P and S) and four subclades, namely P1, P2, S1 and S2 (refs. 7,9). All pathogenic serovars of the previous classification are now included in the P1 subclade, whereas those with intermediate or undetermined pathogenicity are included in the P2 subclade. Saprophytic *Leptospira* serovars are included in the S clade[7].

Although infection can be asymptomatic, symptomatic infections can vary from an uncomplicated acute febrile illness to severe disease characterized by multi-organ dysfunction, including acute renal failure, jaundice (Weil disease) and pulmonary haemorrhage[10,11]. Leptospirosis is commonly diagnosed using the microscopic agglutination test (MAT), point-of-care diagnostics, enzyme-linked immunosorbent assay for anti-*Leptospira* immunoglobulin M (IgM ELISA) or by detecting pathogen nucleic acid sequences[12–14]. However, in resource-limited settings, patients are mostly treated on clinical suspicion without laboratory confirmation. The lack of point-of-care diagnostics with high sensitivity and specificity regularly leads to underdiagnosis and under-reporting, especially in areas with low awareness and endemicity. *Leptospira* spp. are sensitive to a range of antibiotics, including penicillins, cephalosporins, doxycycline, fluoroquinolones and macrolides, and early treatment can be fully curative without complications[15]. However, in patients with leptospirosis who have multiple organ dysfunction, the mortality rate can be as high as 50% (ref. 16).

In this Primer, we summarize advances in our understanding of human leptospirosis and its management. We focus on its epidemiology, pathogenesis, diagnosis, screening and prevention, patient management and effects on quality of life. We further identify knowledge gaps and key challenges that should be prioritized for research in the next decade and beyond.

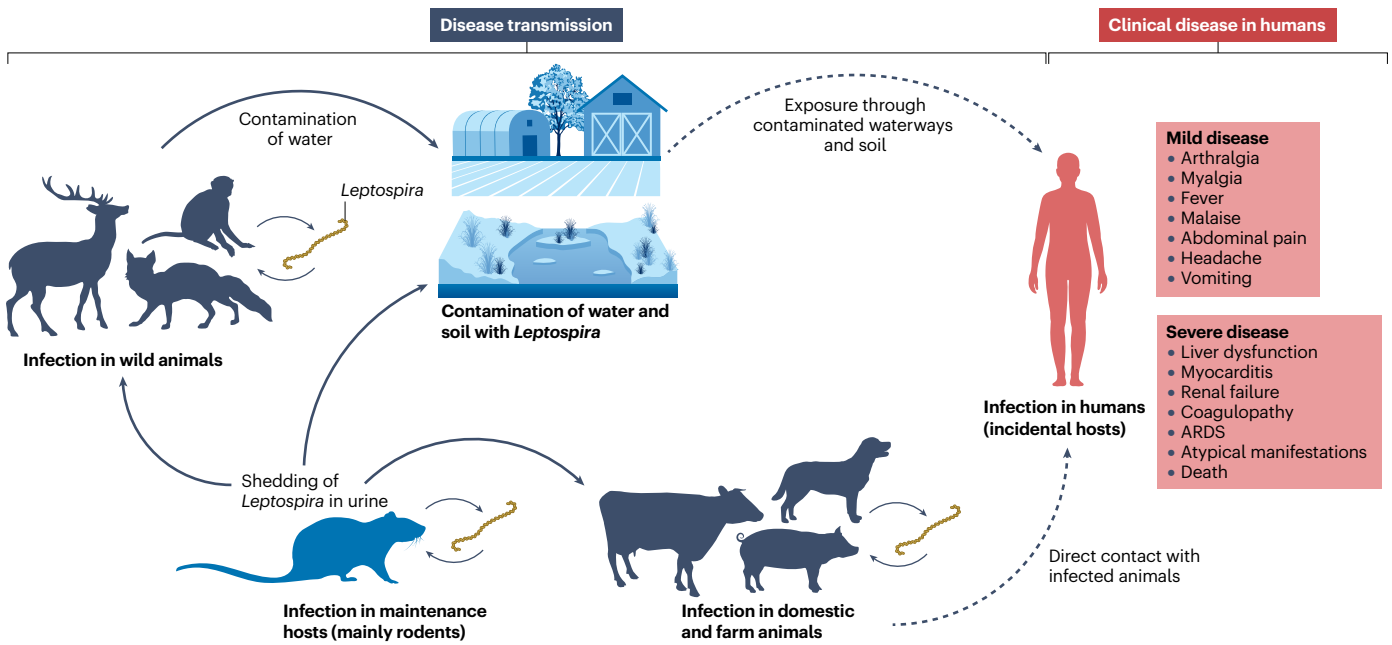

Fig. 1 | Transmission and clinical manifestations of leptospirosis in humans. Leptospirosis is a zoonotic infection affecting a wide range of animals, including mammals. In some animals, such as rats, mice, hedgehogs and marsupials, infections are asymptomatic. These animals, known as maintenance hosts, shed *Leptospira* through their urine, contaminating water and soil, and are a major source of infection in other animal groups (indicated by uninterrupted arrows). Infection in other wild and domesticated animals leads to disease manifestations that are acute (such as in dogs and deer) or chronic (such as in cattle, sheep and goats) and the infection cycle is maintained by direct contact and transmission within herds as well as exposure to contaminated water or soil. Humans are mostly incidental hosts that are infected by direct contact with animals or by exposure to contaminated water or soil. The bacteria enter via breaches in the skin or by penetrating the conjunctivae and mucous membranes. Human-to-human transmission is rare. Once infected, the disease shows a spectrum of manifestations, ranging from asymptomatic infection to symptomatic but uncomplicated disease, to severe leptospirosis characterized by life-threatening organ dysfunction. ARDS, acute respiratory distress syndrome.

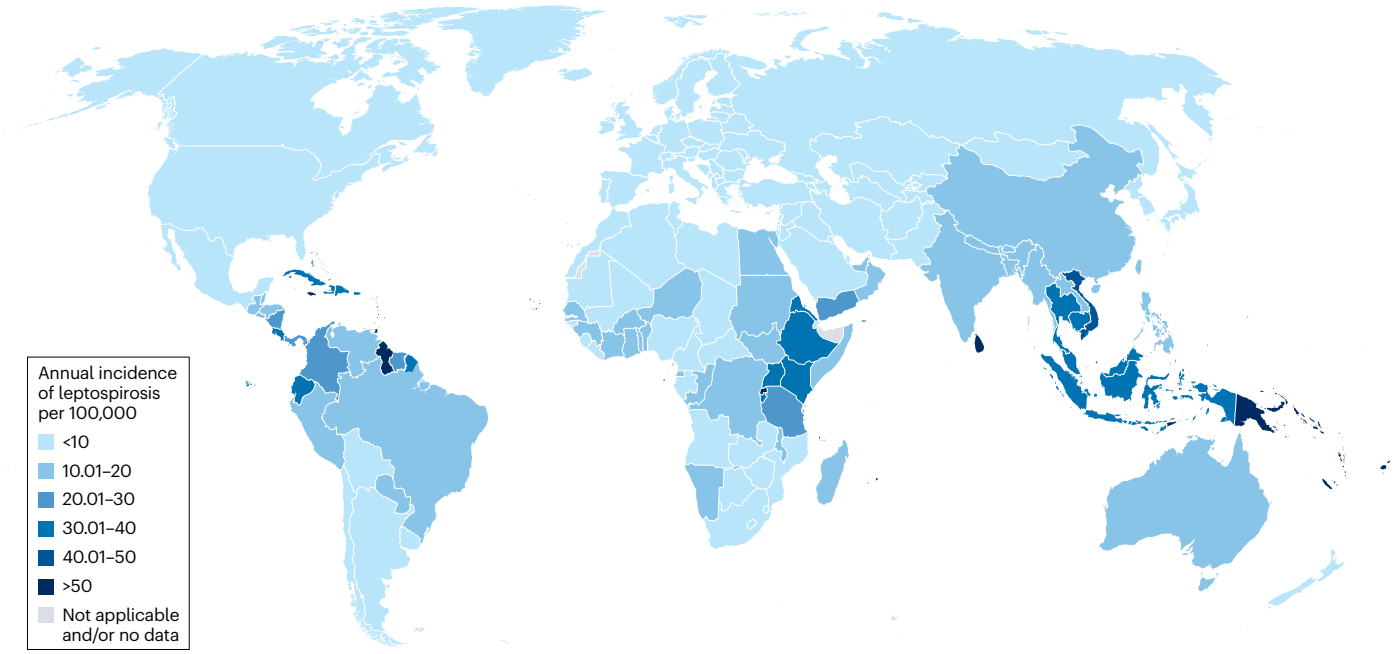

Fig. 2 | Estimated worldwide incidence of leptospirosis. The global estimated incidence (cases per 100,000 population) of leptospirosis per year[10]. Darker colours indicate higher incidence. Countries with the highest rates of leptospirosis morbidity (darkest shade of blue) have a wide disease incidence range from 50 to 300 cases per 100,000 population.

Epidemiology

Incidence and prevalence

Leptospirosis is reported worldwide, with a high disease incidence in low- and middle-income tropical and subtropical countries[10], and a comparatively lower incidence in temperate climates and in high-income countries.

The incidence of leptospirosis in humans is commonly underreported owing to low awareness of the disease, lack of surveillance systems and reference laboratories, and limited access to health care[17,18]. The clinical signs and symptoms are nonspecific and can overlap with other potential differential diagnoses, such as malaria, rickettsioses, dengue or viral haemorrhagic fevers, making a diagnosis challenging in the absence of well-performing rapid diagnostic tests (RDTs) or point-of-care testing. Between 1970 and 2012, 318 outbreaks have been reported globally (36% in Latin America and the Caribbean region, 13% in Southern Asia and 11% in North America), with an average of seven outbreaks annually (range of 1–19) and an overall case fatality rate of 5% (ref. 19). The average outbreak size was 82 cases, although the case number reached as high as 253 in outbreaks in tropical or subtropical regions. However, as mentioned before, a majority of outbreaks might be unreported and these numbers are likely to be an underestimate. In a systematic literature review of 80 studies between 1970 and 2008 across 34 countries, the Leptospirosis Epidemiology Reference Group, an advisory group to the WHO (World Health Organization), estimated that the annual worldwide incidence of leptospirosis was 1.03 million cases (95% CI, 305,000–1,750,000), resulting in 58,900 deaths (95% CI, 23,800–95,800)[10] (Fig. 2). Oceania had the highest estimated annual incidence of leptospirosis, followed by Southeast Asia (150.7 and 55.5 cases per 100,000 population, respectively)[10]. Thus, the mortality rate of leptospirosis is estimated to be more than that of dengue infection (40,500 deaths per year in 2017), which is the main differential diagnosis of concern in the tropics[20]. Most publications after 2015 on leptospirosis epidemiology refer to the data described in the Leptospirosis Epidemiology Reference Group systematic review[10], with no subsequent global update on leptospirosis epidemiology.

Several regional epidemiology updates have been published. Two systematic reviews examined the incidence and prevalence of leptospirosis in Africa from 1930–2014 and 2014–2022, respectively[21,22]. The first review found only seven eligible surveillance and cohort studies to estimate the incidence of leptospirosis, with all except one (in Tanzania) conducted in the Indian Ocean islands of Africa (such as La Reunion, Mayotte and Seychelles)[21]. The estimated annual incidence reported in these 7 studies varied from 3 to 101 cases per 100,000 population. Data for annual prevalence was available from 11 studies, with greater representation from mainland Africa, and the analysis found a prevalence of 2.3–19.8% of acute leptospirosis among hospitalized febrile patients. The second systematic review reported a pooled *Leptospira* seroprevalence of 15% (range of 9–23%) across 18 studies in sub-Saharan Africa that used the MAT for diagnosis[22].

In Europe, an analysis of cases reported to the European Centre for Disease Prevention and Control and the Food- and Waterborne Diseases and Zoonoses Network from 2010 to 2021 identified 12,180 confirmed cases from 23 countries, corresponding to a mean of 0.24 cases reported per 100,000 population per year[23]. The notification rate increased on average by 5% per year over the 12-year period, although approximately 21% of cases were estimated to be imported from outside of Europe.

As for future trends in the next decade, the incidence of leptospirosis is expected to increase as a consequence of climate change, floods and other extreme weather events[24]. This increased incidence

will disproportionately affect lower-income countries owing to a lack of preparedness, inaccessibility to timely diagnosis and treatment, and infrastructure problems (for example, poor drainage after flooding, waste accumulation and sewage contamination) associated with unplanned urbanization[24,25]. Mass migration events triggered by droughts, floods or famines are predicted to further increase incidence.

Disease transmission and risk factors

Leptospira infects humans through mucous membranes (such as in the nose, mouth and eyes) or damaged skin, after humans come into contact with the excreta, body fluids or tissues of infected animals, or contaminated soil or waterways[26–28] (Box 1). A large range of mammals are hosts for *Leptospira* spp., although some of these hosts remain asymptomatic and carry specifically adapted serovars in their renal tubules, which are excreted into the environment for months to years. The infection in animal reservoirs shows natural nidality, with certain serovars being characteristically associated with specific animal hosts[29]. Although leptospirosis is widely perceived as rodent associated, livestock also have an important role in maintaining the transmission cycle of leptospires and infecting humans[30,31].

Box 1 | Exposure risk and at-risk groups

Leptospirosis transmission occurs through direct and indirect exposure to infected animals, and through indirect exposure to contaminated water or soil, under a variety of circumstances.

Direct exposure through infected animals

- Veterinarians
- Abattoir workers
- Farmers
- Hunters and trappers
- Animal shelter workers
- Scientists, researchers and laboratory technicians

Indirect exposure to contaminated water or soil

Occupations

- Agricultural workers: rice field workers, taro farmers, banana farmers, and harvesters of sugar cane and pineapples
- Farming in high-rainfall regions
- Mining
- Sanitation workers
- Military personnel

Recreational activities

- Canoeing
- Kayaking
- Rafting
- Triathlons

Environmental factors

- Poor housing
- Overcrowding in communities
- Poor sanitation
- Flooding

The main infectious host for leptospirosis varies by region and ecological context. For example, in Tanzania, livestock such as cattle have a major role in disease transmission[30]. Conversely, in low-income urban areas, rodents are key contributors to the spread of leptospirosis owing to their close interaction with humans and the contamination of water sources[32]. Depending on the prevalent hosts and *Leptospira* species, serogroups or serovars, the disease patterns in humans can change. Rodent-associated serovars are particularly virulent and are often linked to severe cases of leptospirosis in humans[32].

Humans are incidental hosts for pathogenic *Leptospira* strains[28,33,34]. Many *Leptospira* strains can persist in soil and water under suitable environmental conditions, such as warm temperatures and high humidity. Soil and water serve as important transmission routes for human infection, particularly during periods of heavy rainfall, flooding and agricultural activities[35–37]. Human exposure to *Leptospira* spp. commonly occurs through environmental, occupational or recreational activities[38,39], which in turn are influenced by infrastructure (such as road or bridge access), socioeconomic factors, climate and weather patterns. Rodents have a major role in disease transmission to humans, particularly in low-income settings with poor hygiene. In low-income urban areas, poor sanitation and inadequate drainage can lead to contamination of water supplies with urine from *Leptospira*-infected rodents, and outbreak risks in such settings further increase after flooding or severe weather events such as hurricanes[40,41]. Occupational risks are prominent in farming, slaughterhouse work and other activities involving close contact with infected animals or contaminated environments[42,43]. Recreational exposure arises from fresh water-based activities such as swimming and water sports, and eco-tourism especially in tropical regions where warm, moist conditions facilitate the survival of leptospires in the environment[44].

The prevalence of *Leptospira* spp. varies based on host density, environmental factors such as rainfall; climate (warm and humid conditions); socioeconomic conditions including housing, hygiene and rodent control; agricultural practices such as animal waste management; and vaccination policies. The infection risk is further exacerbated by a lack of awareness, not wearing personal protective equipment (PPE) (for example, boots), unavailability of vaccines or absence of a vaccination policy (for both humans and animals), poor domestic hygiene and sanitation (such as indoor pests and animals, and blocked sewage) and unplanned urbanization[45]. As a result, leptospirosis has a higher incidence in low-income countries and low-middle-income countries[46], and remains associated with resource-poor populations[47–49].

Demographically, men are at a higher risk of infection and death from leptospirosis than women, potentially owing to differences in occupational risk exposure influenced by cultural and gender norms in endemic countries[10]. Compared with other age groups, men 20–29 years of age have the highest risk of infection (relative risk 2.38), whereas men 50–59 years of age have the highest risk of death (relative risk 3.68) from leptospirosis[10]. Infection and death rates by age in women are less well defined, but it is clear that the risk of death is higher among older women (relative risk <0.3 for women below 40 years of age and >0.5 for those over 40 years of age)[10]. Aside from age and sex, mortality is also influenced by exposure risk, socioeconomic circumstances and access to timely diagnostics and treatment[50]. Genetically, the presence of human leukocyte antigen-DQ6 genotype is associated with an increased risk of developing leptospirosis, although the exact mechanisms are unclear[51]. It is difficult to untangle the effect sizes of individual risk factors for leptospirosis morbidity and mortality,

although a statistical model suggested that up to 60% of the variance in leptospirosis case incidence is associated with geographical location, urbanization, life expectancy at birth and living on a tropical island[10].

Mechanisms/pathophysiology

After their entry into the human body, *Leptospira* bacteria are disseminated in the bloodstream (termed leptospiraemia). No lesions are seen at the site of entry, although the site of entry might be through a skin lesion, such as broken skin. Leptospiraemia occurs during the first 8 days of infection, and a higher pathogen load is associated with more severe disease[52]. Direct damage caused by pathogenic *Leptospira* and adverse immune responses induced by pathogen and host factors contribute to disease pathogenesis[5]. Although the pathophysiology of severe leptospirosis is not fully understood, it is widely accepted that host immune dysregulation, leading to hypercytokinaemia (cytokine storm), has a key role[53].

Histopathology

Common histopathological findings in severe leptospirosis include localized or generalized endothelial damage in capillaries, which leads to exudate formation and extravasation of red blood cells, lymphocytes and granulocytes in affected organs[54–56]. The liver and kidneys are the most commonly affected organs. In the liver, intrahepatic cholestasis, Kupffer cell hypertrophy and hyperplasia[57,58], sinusoidal congestion, enlargement of the space of Disse (space between a hepatocyte and the adjacent sinusoid), and inflammation of liver parenchyma with focal necrosis are observed[59], which clinically manifests as jaundice (hyperbilirubinaemia) and abnormal liver function tests. In the kidneys, interstitial nephritis with tubular inflammation and necrosis are typically seen[59–61]. Lung involvement is a less common complication, with histopathological changes that include alveolar and interstitial inflammation and haemorrhage mostly seen in patients with severe leptospirosis[62]. Systemic dysfunction of coagulation pathways and interstitial myocarditis can also contribute to pulmonary haemorrhage and oedema[63,64], and *Leptospira* can infiltrate pulmonary tissue[65].

Molecular and cellular pathology

In mild forms of leptospirosis, it is unclear to what extent disease manifestations are attributable to direct invasion by the pathogen as opposed to the host immune response. Mild illness is typically described as biphasic, with an acute (leptospiraemic) phase, during which the pathogen can be isolated from blood, followed by an immune phase, during which the pathogen load in tissues declines and the host immune-response-driven organ damage predominates. However, these two phases are not always mutually exclusive, as leptospires can still be isolated from affected organs during the immune phase.

The earliest inflammatory response following pathogen entry is triggered by recognition of microorganism-associated molecular patterns, including LPS, lipoproteins and nucleic acids, by membrane-bound or soluble pattern recognition receptors (such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs)) on macrophages and neutrophils[66] (Fig. 3). Although TLR4 is often the key receptor that detects LPS in Gram-negative bacteria, TLR2 is preferentially activated in leptospirosis[67,68] and, in combination with TLR1, can effectively recognize *Leptospira* lipoproteins and peptidoglycans[66]. Single-nucleotide polymorphisms identified in TLR1 (Ile602Ser) and TLR2 (Arg753Gln) increase susceptibility to leptospirosis infection in humans by 4-fold and 11-fold, respectively[66]. Stimulation of TLRs or NLRs activates signalling through mitogen-activated protein kinases (MAPK), as well as activation of other transcription factors such as nuclear factor-κB and activator protein 1 (ref. 69).

Activation of transcription factors increases the expression of genes encoding pro-inflammatory cytokines (such as tumour necrosis factor (TNF), IL-8, IL-6 and IL-1β) and anti-inflammatory mediators (IL-10, transforming growth factor-β (TGFβ), IL-4 and IL-13)[70]. The relative concentration of these cytokines, as well as their timing of production and kinetics, must be tightly controlled to mount a successful immune response. In addition to the above-mentioned molecular signalling pathways, haemolysins secreted by the pathogen can also independently induce the production of pro-inflammatory cytokines[71]. Unregulated production of TNF, IL-6 and IL-10 seems to be the initial driver of the cytokine storm in severe leptospirosis, which subsequently induces immunoparesis via high levels of anti-inflammatory cytokines such as IL-10 and TGFβ[72] (Fig. 3). Thus, in severe disease, the levels of both pro- and anti-inflammatory cytokines are increased compared with in mild disease[73]. However the ratio of IL-10 to TNF is higher in severe disease and dominance in IL-10 is thought to supress the protective T helper 1 cell-dominated adaptive immune response leading to immunoparesis[72,73].

Upon activation of cell surface receptors, macrophages engulf both opsonized and unopsonized leptospires, whereas neutrophils are capable of engulfing only those that have been opsonized with serovar specific antibodies[74–76]. In macrophages, opsonization status can determine the fate of the engulfed *Leptospira*, with opsonized leptospires being confined to phagolysosomes, whereas unopsonized leptospires are found in the cytosol, where they replicate[77]. Effective phagocytosis is followed by elevated production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in oxygen-dependent killing. However, leptospires can catalyse ROS breakdown by oxidoreductases, promoting their resistance to ROS-induced killing inside macrophages[78]. Moreover, the unregulated excess production of ROS and RNS results in oxidative stress and tissue damage[79].

Unlike macrophages, neutrophils fail to engulf pathogenic *Leptospira* in vitro, even in the presence of normal serum, indicating that opsonization by complement alone is not sufficient, and specific antibodies are required to be present. Thus, neutrophils might not be effective against pathogenic serovars in non-immune individuals[80]. The mechanisms underlying this ability of pathogenic *Leptospira* to evade neutrophils are not known. By contrast, neutrophil degranulation might be an effective defensive mechanism, as pathogenic *Leptospira* spp. are sensitive to both primary and secondary granule contents. Myeloperoxidase, a lysosomal enzyme in neutrophils, is one of the most effective microbicides against leptospires[81,82].

Adaptive immunity against extracellular *Leptospira* is mainly a humoral (antibody-mediated) response. Anti-*Leptospira* antibodies are protective because passive immunization offers protection, and opsonization by anti-*Leptospira* immunoglobulin G (IgG) triggers complement activation, leading to increased phagocytosis[76,80,83]. Anti-*Leptospira* antibodies produced after natural infection can last for as long as 7–8 years post-infection[84], although it is unclear whether antibodies give cross-protection against other serovars[85]. The T cell response against *Leptospira* is poorly understood, but interestingly, the proportion of γδ receptor T lymphocytes, usually a minor lymphocyte subpopulation compared with αβ-receptor T cells, are elevated in the peripheral blood of patients with acute leptospirosis[86]. The cytokines IL-4 and IL-13 mediate a dominant T helper 2 cell response, diverting resources away from T helper 1 cells, which are more important for

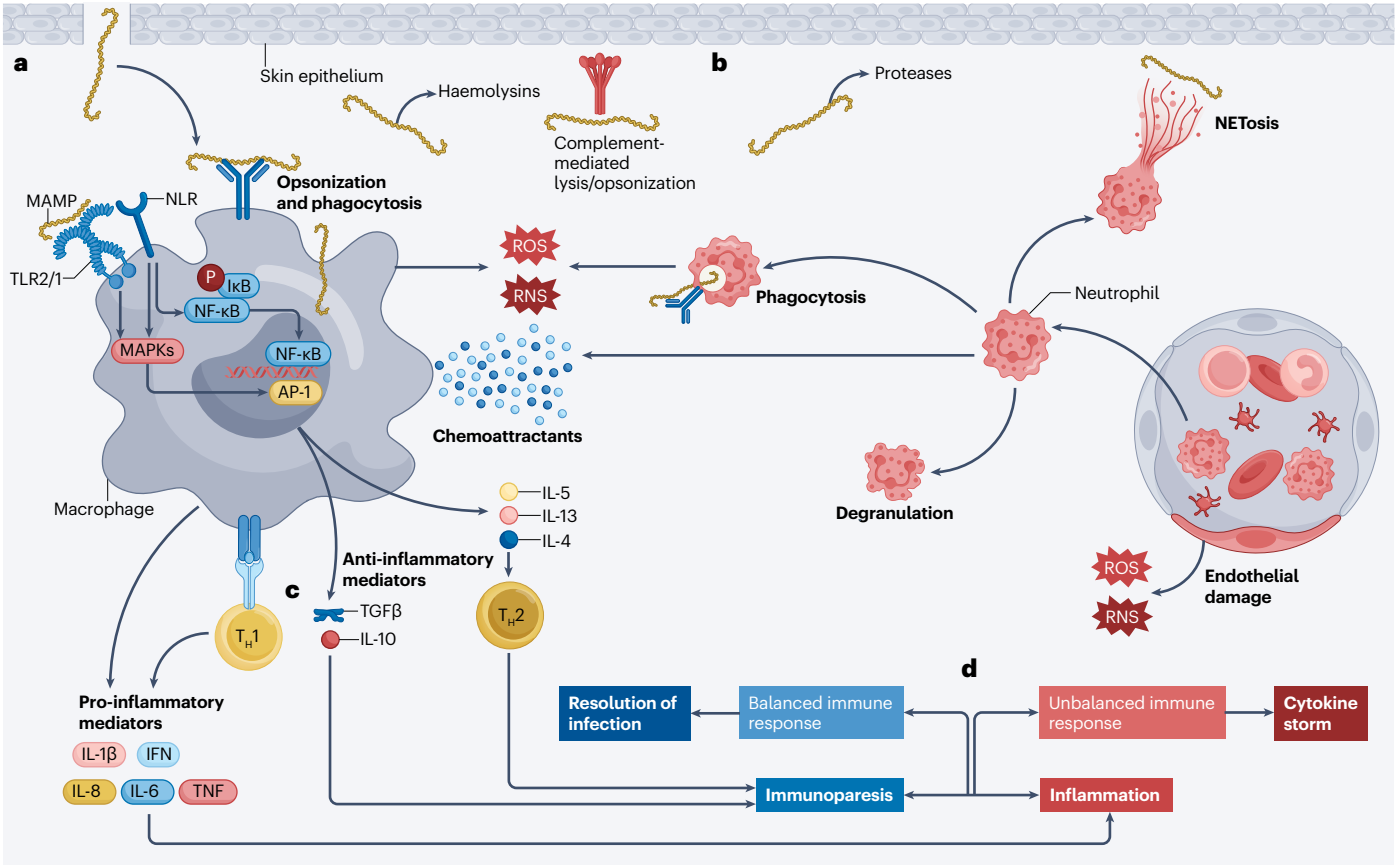

Fig. 3 | The pathogenesis of severe leptospirosis. a, Pathogen entry and recognition by tissue-resident macrophages. *Leptospira* enter through breaches in skin, and their microorganism-associated molecular patterns (MAMPs) are recognized by macrophages via pattern recognition receptors, including toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs). Activation of these receptors triggers downstream signal transduction pathways, activation of transcription factors (nuclear factor κB (NF-κB) and activator protein 1 (AP-1)), and finally inflammatory cytokine and chemokine production through gene upregulation. **b**, Recruitment of other innate immune cells, including neutrophils. Pro-inflammatory cytokines and chemokines produced by tissue-resident macrophages induce the extravasation of neutrophils and other immune cells, leading to expression of both pro-inflammatory (tumour necrosis factor (TNF)), IL-8, IL-6 and IL-1β) and anti-inflammatory (IL-10 and transforming growth factor-β (TGFβ) mediators. Innate immune mechanisms kill spirochaetes through complement-mediated lysis and by neutrophil- or macrophage-mediated phagocytosis. Macrophage phagocytosis is more effective following opsonization with anti-*Leptospira* antibodies. Neutrophils can also kill *Leptospira* via degranulation and neutrophil extracellular traps (NET)osis. Trafficking of *Leptospira* into phagolysosomes after phagocytosis leads to release of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which, if excessive, leads to host tissue damage. Host endothelium can be damaged by direct interactions with *Leptospira* and as a result of *Leptospira* toxins, as well as inflammatory cytokines produced by the host. ROS and RNS further exacerbate the inflammatory response. Extracellular release of neutrophil DNA structures or NETosis can also trap *Leptospira*, providing a physical barrier against their spread. **c**, Activation of adaptive immune responses. Antigen presentation by macrophages and dendritic cells activates T helper 1 (T_H1) and T_H2 cells, as well as regulatory T cells. The pro-inflammatory effect of the T_H1 response is balanced by anti-inflammatory mediators produced by regulatory T cells (IL-10 and TGFβ), and also by diverting resources to the T_H2 response pathway (through IL-4, IL-5 and IL-13 production). **d**, A well-balanced immune response between pro-inflammatory and anti-inflammatory responses kills spirochaetes and then is effectively terminated when no longer needed. An unbalanced immune response triggered by increased pathogen virulence, impaired kinetics of the immune response, comorbidities or genetic predisposition of the host, leads to overproduction of both pro-inflammatory and anti-inflammatory cytokines, culminating in a cytokine storm that can have downstream consequences of multi-organ dysfunction and potentially death. IFN, interferon; IκB, inhibitor of nuclear factor-κB; MAPK, mitogen-activated protein kinase.

antibacterial cellular immunity[72]. Pathogenic *Leptospira* spp. have a direct effect on the interaction between myeloid-derived dendritic cells and T helper cells, activating regulatory T cells that potentially leads to immunoparesis[87]. Overall, the actions of pro-inflammatory and anti-inflammatory cytokines, the kinetics of their production and release, their protagonist or antagonist interactions, as well as other risk factors associated with the host (such as comorbidities, immunosuppression, genetic polymorphisms and timely treatment) seem to determine the diverse clinical manifestations of leptospirosis.

Genetic polymorphisms and illness phenotypes

Evidence for host genetic polymorphisms that affect leptospirosis disease severity is limited to small case–control studies examining associations with candidate gene pathways, which are inherently biased

towards immune pathways associated with pathogenesis. Polymorphisms within the genes encoding IL-1β, TLR, IL12RB1, multiple cytokines inducible SH2-containing protein (CISH), IL-4 and in the promoter of the macrophage migration inhibitory factor (MIF) gene, have been associated with increased susceptibility to leptospirosis[88–90]. Alleles of human leukocyte antigen classes I (A and B) and II (DQ6) loci are also associated with susceptibility to infection[51,90]. Furthermore, polymorphisms within TLR genes are also associated with the likelihood of organ dysfunction in symptomatic individuals[66]. Genome-wide association studies or whole-genome sequencing studies are needed to obtain a more detailed understanding of individual genetic susceptibility to leptospirosis.

Infectivity and virulence

The corkscrew motility of *Leptospira* confers the unique ability to penetrate viscous environments, such as connective tissue[91]. *Leptospira* are thus able to enter breached skin, penetrate the endothelium and disseminate through the bloodstream.

Some researchers have proposed that virulence modifying proteins, encoded by the leptospiral PF07598 gene family, are exotoxins[92], although their exact role in disease pathogenesis is unclear[93]. The immunopathogenesis of leptospirosis is driven by a concerted interaction between the host immune system, bacterial LPS and OMPs. Some genes coding for OMPs such as *lipl32* and *lig* (*Leptospira* immunoglobulin-like protein genes) are only present in pathogenic *Leptospira* spp., rather than non-pathogenic species, and their encoded OMPs are believed to be important for tissue invasion and host cell adherence. Of these, *LigB* is present in all known pathogenic *Leptospira* spp., whereas *LigA* and *LigC* are found only in some pathogenic serovars[94]. The OMP LipL32 inhibits myeloperoxidase activity in vitro, resulting in diminished neutrophil function and enhanced inflammation, which lead to oxidative stress[81]. Loa22 is another OMP that forms complexes with *Leptospira* peptidoglycan (LPGN), which in turn interacts with TLR2 to induce the pro-inflammatory cytokines IL-8, MCP1 and TNF via downstream signalling[74]. OmpL37 is also a protein expressed only by pathogenic serovars and has high affinity to human elastin, fibronectin and fibrinogen, and thereby probably mediates virulence by aiding tissue adherence[95].

Pathogenic *Leptospira* also avoid immune destruction by interfering with host complement pathways, including classical, alternative and lectin pathways. *Leptospira* metalloproteinases prevent complement protein accumulation on their surface, thus inhibiting the formation of the membrane attack complex that can kill the pathogen by disrupting its cell membrane[96]. Mechanistically, accumulation of plasminogen on the surface of the bacteria seems to lead to degradation of complement components C3b, C4b and C5 by plasmin, thereby preventing the progression of the complement cascade. Surface binding of complement regulators such as factor H, C1 esterase and factor H-like protein 1 affects the alternative pathway via C3b inactivation, and binding of factor I causes C4b inactivation, thereby affecting both the classical and lectin pathways[97]. Finally, pathogenic *Leptospira* can interfere with human adaptive immunity by limiting the capacity of human monocyte-derived dendritic cells to activate $CD4^+$ T cells and instead induce regulatory T cells[87]. However, given the difficulty in culturing *Leptospira* directly from patients infected with severe disease, much of the above evidence on pathogen virulence is from in vitro studies and animal models (for example, hamsters and mice), which might not be directly translatable to human disease.

The genomes of *Leptospira* spp. are approximately 3.9–4.6 Mb in size[98]. Whole-genome sequencing has revealed differences between pathogenic and saprophytic species, leading to the identification of putative virulence-determining proteins such as haemolysins and adhesion molecules. For example, pathogenic *L. interrogans* has an orthologue of a haemolysin gene that is a known virulence factor in another spirochaete species[5,99]. The sphingomyelinase C gene (*sphA*)[100], its homologue *sphH* and a third gene *sph2* (ref. 101) are other genes that might potentially encode proteins that determine virulence by mediating cytotoxic properties, haemolysis and/or endothelial damage[5].

Cytokine storm and immunoparesis in severe disease

Severe leptospirosis is probably the result of multiple, interacting pathogenic mechanisms (as described above). In genetically, demographically and clinically susceptible individuals, leptospiraemia will typically last 7–10 days after symptom onset, owing to a combination of pathogen virulence and an impaired immune response. This leptospiraemia is likely to trigger the unregulated production of pro-inflammatory cytokines such as TNF and IL-6, as well as ROS- and RNS-mediated host tissue damage, which then induces the unregulated production of immune modulators such as IL-10 and TGFβ, resulting in a cytokine storm[72,73]. Thus, in severe disease, the levels of both pro- and anti-inflammatory cytokines can be increased, but owing to their dysregulated production and altered kinetics, the immune response is destructive rather than protective (Fig. 3).

Diagnosis, screening and prevention

Clinical disease

Asymptomatic infection. Several reviews on human leptospirosis state that most infections are asymptomatic, but with limited evidence[2,102,103]. In Malaysia, screening of 303 asymptomatic sanitation workers using PCR and the MAT revealed a positivity rate of 22% and 44%, respectively, for each diagnostic test. Genomic sequencing revealed that all PCR-positive samples had pathogenic or intermediate pathogenicity strains of *Leptospira*[47]. Similarly, of 314 asymptomatic people living in the rural Peruvian Amazon, 59% were seropositive by the MAT but only 4% had *Leptospira* DNA in their urine, based on 16S ribosomal RNA gene detection[104]. Again, genomic sequencing identified both pathogenic and intermediate strains. In Nicaragua, cross-sectional sero-surveillance of 566 people living in a leptospirosis outbreak area revealed anti-*Leptospira* IgM antibodies in 85 individuals (15%), indicating recent infection, although only 25 of them reported a febrile illness during the preceding 2 months[105]. In a cross-sectional population survey in urban and semi-urban areas in Sri Lanka, 269 (33.2%) of 810 individuals screened showed positivity for a pathogenic serovar by the MAT at a titre of 1:40 or more[106]. Overall, the data on asymptomatic infection in humans are sparse and limited to small studies in high-risk settings. Collating data on asymptomatic human infections is inherently difficult because these individuals do not present to health-care workers. Furthermore, even if they do present to health-care workers, such as during screening of high-risk groups, they are likely to be presumptively treated or offered prophylaxis after a clinical risk assessment, given the difficulties in confirming a leptospirosis diagnosis. On the contrary, there are many animal studies showing that the majority of infections are asymptomatic in rodents, as well as in farm and domesticated animals such as dogs, sheep and cows[107–109], although such observations cannot be extrapolated to humans because of species-dependent susceptibility to infection[110].

Symptomatic infection. Symptomatic leptospirosis can be anicteric or icteric (with or without jaundice) and manifests after an incubation

period of 2–30 days[103]. Icteric infection, termed Weil disease, is at the more severe end of the spectrum, and is associated with a higher risk of multi-organ failure and death.

In the acute phase of leptospirosis, patients typically present with an acute onset febrile illness characterized by high fever, chills and rigors, myalgia, nausea, vomiting and headache (Fig. 1). Examination might reveal a prostrate patient with conjunctival erythema or subconjunctival haemorrhages, hepatomegaly, splenomegaly, lymphadenopathy, muscle tenderness and a skin rash (maculopapular or urticarial) that can be difficult to detect in people with darker complexions[2,111]. Conjunctival suffusion is characteristic of leptospirosis and is not commonly seen in other infections. Moreover, polyuria is commonly observed and is thought to result from impaired function of proximal convoluted tubules in nephrons[112].

The progression of illness is continuous in most patients. Less commonly, in patients who experience a biphasic (leptospiraemic and immune phases) illness, a period of 'remission' (3–4 days), followed by return or exacerbation of symptoms, is observed. *Leptospira* can still be detectable in urine during the immune phase but not in blood[2]. A minority of patients experience an extended immune phase characterized by aseptic meningitis or uveitis that can last up to a month.

Although anicteric disease is typically mild, complications such as acute cholecystitis and pulmonary haemorrhage can occur in these patients, albeit rarely. Early icteric leptospirosis has the same symptoms as anicteric disease, with the addition of jaundice (Fig. 4). In severe disease, the kidneys, liver, lungs, brain and heart are most often affected[111]. Acute kidney injury can be of rapid onset, characterized by reduced urine output, haematuria, generalized oedema, red blood cell casts on urine analysis and rapidly rising serum creatinine with or without hypokalaemia[113]. Liver involvement manifests as hyperbilirubinaemia and elevation of hepatic transaminase levels[114]. Lung involvement is externally manifested as tachypnoea, tachycardia, non-productive cough, dyspnoea with fine or coarse crackles on auscultation[102], and critically ill patients with pulmonary involvement can have severe pulmonary haemorrhagic syndrome leading to respiratory failure[115]. Myocarditis in leptospirosis can initially manifest as nonspecific electrocardiographic changes before rapidly progressing to myocardial hypokinesia and reduced ejection fraction on echocardiography, with vulnerability to fatal arrhythmias[63]. A systematic review of mortality rates of untreated leptospirosis found an overall median mortality of 2.2% across 41 patient series, that increased to 20% (range 0–40%) in patients with icteric disease[116]. Acute kidney injury and pulmonary haemorrhage are the most common immediate causes of death in leptospirosis[117]. In addition to the above symptoms, leptospirosis can sometimes present with atypical manifestations, which leads to major diagnostic delays (Box 2).

Diagnosis

Diagnostic tests. Leptospirosis can be diagnosed with direct or indirect methods. Direct diagnostic methods observe the intact pathogen (using culture and microscopy) or observe its genome or antigens (through sequencing or immunological assays). Indirect methods, by contrast, detect pathogen-specific immune responses, such as antibodies, in the host triggered by a recent infection (Fig. 5).

Direct visualization of *Leptospira* bacteria with dark-field microscopy has low sensitivity and specificity, and accordingly is rarely used in routine diagnosis[118]. *Leptospira* can be cultured from body fluids (such as blood, urine or cerebrospinal fluid), although specific medium (Ellinghausen–McCullough–Johnson–Harris medium) is required and growth can take several weeks, meaning that this approach does not help in making treatment decisions[119,120]. Although antibiotic sensitivity testing is only possible by culturing bacteria, it is unhelpful in clinical management as antibiotic administration cannot be delayed and *Leptospira* are sensitive to many empirically administered antibiotics.

Immunological diagnostics are the most commonly used tests to confirm infection and can be direct (antigen detection) or indirect (anti-*Leptospira* antibody detection). The presumed but contested gold-standard diagnostic approach for detecting leptospirosis is the MAT, which uses a panel of different pathogenic *Leptospira* serovars to detect binding antibodies (IgG and IgM) in patient serum[121]. With the MAT, serum samples taken 4–6 weeks apart from acute and convalescent patients are tested to detect a greater than fourfold rise in antibody titres or, to confirm current infection, a single high baseline titre above a pre-determined cut-off (or seroconversion) is investigated[121]. Similar to culture methods, the MAT is useful to characterize the at-risk population exposure in epidemiological studies, including serovar diversity[122]. However, the MAT is cumbersome to perform (often limited to reference laboratories), with the need to maintain a live *Leptospira* spp. panel, based on the regional serogroup prevalence. Therefore, less well-equipped laboratories rely on diagnosing acute infection with locally validated ELISA or immunoblot for anti-*Leptospira* IgM. Such assays have been designed to detect antibodies against several OMPs, including LipL32, LigA and B, Lp29, Lp49, LipL41, OmpL1 and Loa22. A meta-analysis of the effectiveness of ELISA for leptospirosis diagnosis reported a combined sensitivity and specificity of 78–86% and 90–91%, respectively[123,124]. However, IgM ELISA has many limitations, including lower sensitivity in early infection compared with nucleic acid detection[125], low specificity owing to the persistence of antibodies after

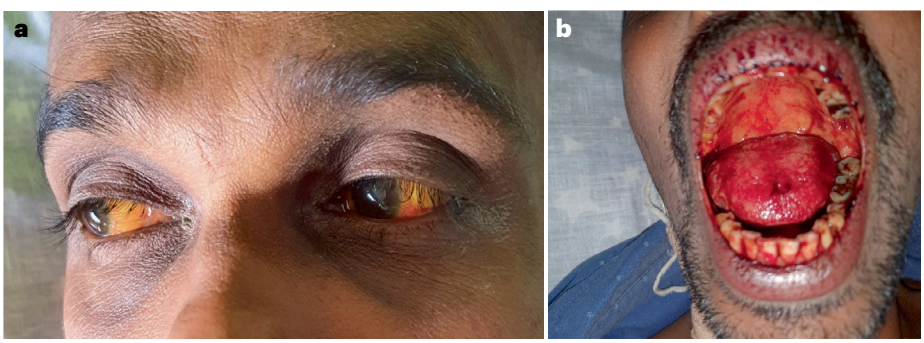

Fig. 4 | Jaundice, conjunctival suffusion and haemorrhage in icteric leptospirosis. a, Jaundice with conjunctival suffusion is characteristic of leptospirosis. **b**, Bleeding can occur due to coagulopathy; bleeding from the gums is shown here.

acute infection[126], variability in sensitivity and specificity across countries or regions[127], cross-reaction with antigens of other pathogens or different *Leptospira* serogroups or serovars[128], and being too expensive for routine use. Point-of-care RDTs for anti-*Leptospira* IgM are therefore becoming popular in resource-limited settings[12,13,129]. RDTs are relatively inexpensive, commercially produced and easy to use; however, the performance of RDTs is lower than laboratory techniques (MAT or ELISA), with sensitivities ranging from 51% to 69% (ref. 130). MAT is typically considered as a gold standard when determining the performance of other diagnostic tests including RDTs, but this approach is somewhat flawed given that researchers have cast doubt on the validity of using the MAT as the immunological gold standard[13]. Studies that assessed RDTs using Bayesian latent class modelling alongside the MAT (instead of using the MAT as the gold standard), reported that RDTs were probably more sensitive but less specific compared with MAT[129,131]. RDTs capable of detecting *Leptospira* antigens, which would facilitate early diagnosis, are not yet available. In general, future research should validate the performance of ELISA and RDT for specific regions and populations before their use as diagnostic tools.

Genomic diagnosis techniques detect *Leptospira* nucleic acids, although they are subject to false positives (detecting dead bacteria in a resolved infection) or false negatives (low bacteraemia in the late symptomatic phase). Furthermore, in resource-limited settings, genomic sequencing is expensive and less accessible, and therefore alternative methods for confirming the presence of *Leptospira* genomes should be considered, such as quantitative PCR (qPCR), gel electrophoresis, restriction enzyme analysis or direct visualization of products after loop-mediated isothermal amplification (such as by a colorimetric reaction)[14,132]. In particular, qPCR targeting the *lipL32* gene is highly sensitive and specific and is often used in research and diagnostics[133]. With advances in next-generation sequencing and third-generation (long-read) sequencing, sequencers are becoming smaller, easier to use, more affordable and less reliant on sophisticated laboratory infrastructure for their operation[134]. This could make genomic sequencing of *Leptospira* more accessible.

Extracting all genomic material (DNA and RNA) from clinical specimens for ultradeep sequencing (clinical metagenomics or meta-transcriptomics)[135], or the use of commercially designed probe panels that bind all known pathogenic viruses and bacteria, are increasingly utilized in research to diagnose infections, including leptospirosis[136]. These cutting-edge methods are slowly making their way into clinical settings for diagnosing febrile illnesses of unknown origin, complementing traditional pathogen-specific tests.

Differential diagnosis. In resource-limited settings, routine patient management does not include laboratory confirmation of leptospirosis, as confirmatory tests (described above) can be time consuming (except for RDTs) and expensive, vary in their accuracies and/or are often not readily accessible[129,130]. A clinical diagnosis based on history of exposure and clinical features, supported by readily available non-confirmatory laboratory tests and imaging, is often made before starting treatment. Exposure risk is one of the most important predictors of leptospirosis[137]. Ruling out dengue infection and malaria should be prioritized when all three conditions are endemic in the community. Malaria can be ruled out using RDTs and by performing a blood smear examination. However, ruling out dengue infection, even with point-of-care diagnostics (such as the NS1 dengue antigen test) is more difficult given the varying sensitivity across the period of illness, false positives and cost restraints for obtaining test kits in resource-limited settings[138,139]. The full blood count may offer few clues in differentiating leptospirosis from dengue as neutrophilia is observed in the former whereas lymphopenia with a relative lymphocytosis is often observed in the latter. Thrombocytopenia is seen in both infections, but it is more common and accompanied by plasma leakage in dengue infection[138,140,141]. The urine in leptospirosis will typically contain red and white blood cells above the reference range (or their casts), whereas in patients with dengue fever, typically no abnormalities are detected, except for haematuria in some patients with bleeding manifestations. Although serum levels of liver transaminases can be elevated in both infections[114], hyperbilirubinaemia is often present in leptospirosis but is rare in dengue infection. Importantly, if leptospirosis is suspected, the patient must be observed for reduced urine output, which will precede a rise in serum creatinine. Even a moderate rise in serum creatinine can indicate a substantial drop in glomerular filtration rate. If serum creatinine is rising, the patient's potassium levels must be monitored during illness and recovery. It is also important to monitor cardiac function with serial electrocardiograms and echocardiography. Obtaining a baseline chest radiograph is useful for comparing with later radiographs if the patient develops pulmonary symptoms. In addition to the above, further tests might be needed, depending on the clinical status of the patient (such as clotting profile, cardiac troponin levels, and cerebrospinal fluid analysis).

Box 2 | Atypical manifestations of leptospirosis

Although atypical manifestations of leptospirosis are rare, their absolute number is higher in countries/regions where the overall disease incidence is high, contributing to diagnostic delays and preventable deaths[230]. Atypical presentations include gastrointestinal (acute pancreatitis and cholecystitis)[231,232], haematological (autoimmune haemolytic anaemia, disseminated intravascular coagulation, haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura)[140,233–235], neurological (encephalitis, Guillain–Barre syndrome, transverse myelitis, acute disseminated encephalomyelitis, hydrocephalus with raised intracranial pressure, intracranial bleeding and thrombosis, cerebellar symptoms and mononeuritis multiplex)[236–243] and ophthalmological (uveitis and optic neuritis)[244,245] manifestations. Furthermore, leptospirosis in pregnancy is associated with miscarriage, stillbirth, post-partum haemorrhage and potential transmission of infection to the fetus (no conclusive evidence)[246–249]. The bacteria might also survive for longer in immunosuppressed people or in immunologically privileged sites such as the eye in immunocompetent patients[110].

Clinical risk prediction

Clinical risk prediction for leptospirosis often occurs in one of two settings: to identify patients that are likely to have leptospirosis (in the absence of diagnostic testing) or to identify patients that already have leptospirosis but are likely to progress to severe leptospirosis (for triaging).

Clinical risk prediction for possible leptospirosis. Given the difficulties in obtaining a timely laboratory-confirmed diagnosis, scoring systems based on clinical symptoms and ancillary investigations have

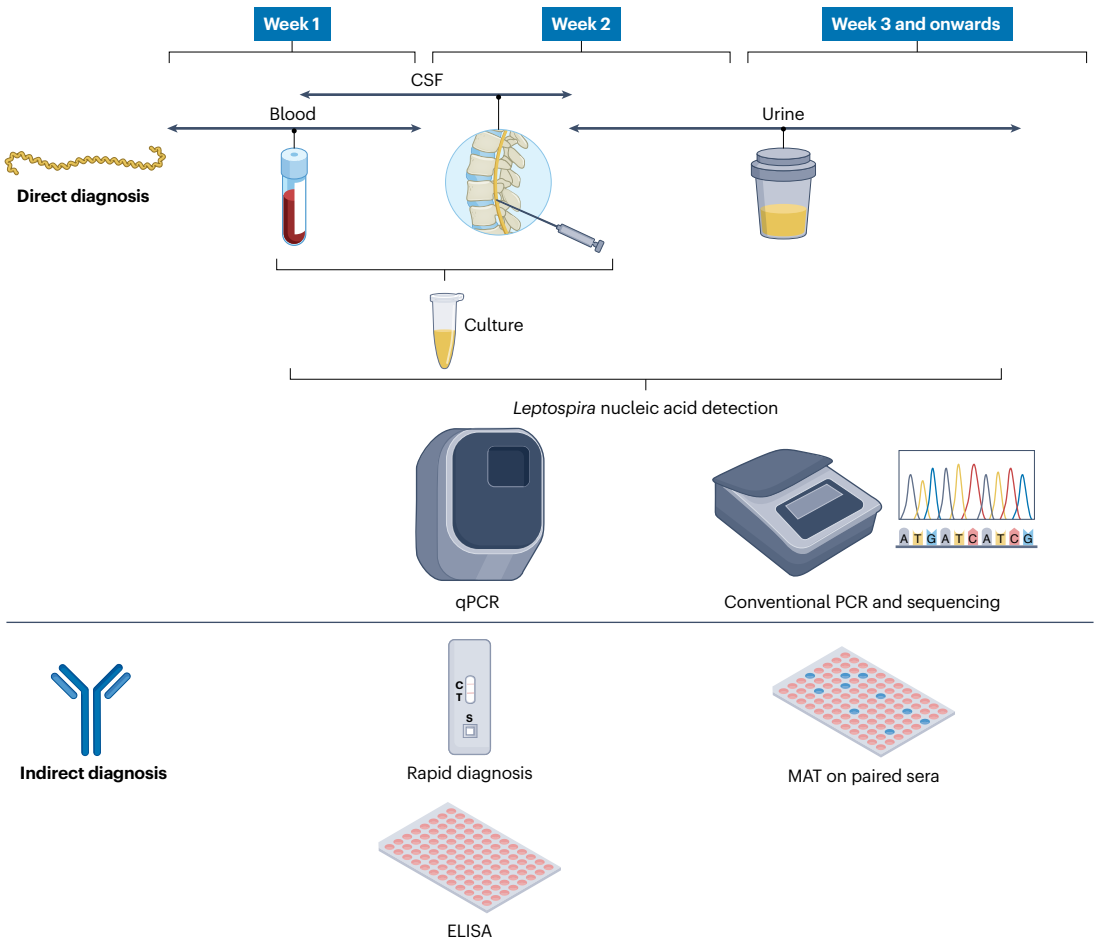

Fig. 5 | Laboratory diagnosis of leptospirosis. Leptospirosis can be detected by direct or indirect means. Indirect diagnosis involves detecting the immunological response against the pathogen, whereas direct diagnosis involves demonstrating the presence of the pathogen by culture or its genomic material through PCR. Weeks 1, 2 and 3 refer to the symptomatic phase only (excluding the incubation period). For direct diagnosis, *Leptospira* are typically detected in the blood within week 1, and in cerebrospinal fluid (CSF) up to day 10. For confirming infection by culture, the sample for culture is often collected within the first 2 weeks of symptoms and culturing takes place over several weeks. Urine samples are typically used for *Leptospira* nucleic acid detection by quantitative PCR (qPCR) or conventional PCR, followed by genomic sequencing. For indirect diagnosis, anti-*Leptospira* immunoglobulin M (IgM) is typically detectable between days 6 and 10 and reaches a plateau by week 3. Early IgM response can be detected by rapid diagnostic tests or enzyme-linked immunosorbent assay (ELISA). The microscopic agglutination test (MAT) requires paired sera obtained at least 1–2 weeks apart to demonstrate a clinically relevant (fourfold or greater) rise in antibody titre. A single sample might be considered positive if the initial titre is very high (for example, >1/400). The pictorial depiction here of rapid diagnostics, ELISA and MAT does not imply that they must be done in the same sequence.

been designed to predict the likelihood of a patient having leptospirosis. In a single-centre prospective study in Sri Lanka, a scoring system was developed that uses history of exposure, serum creatinine and bilirubin levels, platelet count and differential neutrophil percentage as independent predictors of having laboratory-confirmed leptospirosis, which had a sensitivity of 80.3% and a specificity of 60.2% to predict confirmed infection[137]. In a similar multicentre prospective study in Thailand, a scoring system was developed that is based on seven predictors (hypotension, jaundice, muscle pain, acute kidney injury, low haemoglobin, hypokalaemia or hyponatraemia, and neutrophilia), which had a sensitivity of 73.5% and a specificity of 73.7% (ref. 142). In a third study (from Thailand), a scoring system was derived that is based on history of exposure, urine dipstick positivity for protein and blood, and neutrophil count, which had a sensitivity of 72.4% and specificity of 61.7% (ref. 143). Overall, the sensitivities and specificities of these scoring systems are too low for the reliable exclusion of patients as not having leptospirosis. Furthermore, the independent variables associated with a diagnosis of leptospirosis were not similar across these studies, which suggests that such predictors may be site specific and cannot be generalized. Therefore, developing accurate and more cost-effective point-of-care diagnostic tests remains a priority.

Risk prediction for severe disease (outcome prediction). Several systematic reviews[117,144] and original research studies have attempted to identify risk factors for death or organ dysfunction (such as acute kidney injury) in severe leptospirosis. SPiRO is a four-point score (0–3) developed from a retrospective analysis that identified hypotension, abnormal auscultatory findings and oliguria on admission

as independent predictors of severe leptospirosis[145]. A second retrospective study derived a new predictive score (Quicklepto) using age <40 years, mean arterial pressure <80 mmHg, haematocrit <30%, lethargy and pulmonary involvement as independent predictors for in-hospital death, which seemed to outperform SPiRO (area under the receiver operating characteristic curve of 0.5 versus 0.788)[146]. A third single-centre prospective study identified high serum potassium and creatinine levels, a high respiratory rate, shock on presentation and a lower Glasgow coma scale score as independent predictors for risk of pulmonary haemorrhage (area under the receiver operating characteristic curve of 0.94)[147]. Unfortunately, these and multiple other studies[148–150] that have characterized determinants of disease severity have poor concordance of findings, which limits their generalizability. Furthermore, these tools were derived from patients with confirmed leptospirosis, whereas a clinically useful predictive tool should consider all clinically suspected patients, particularly given that a diagnosis is often not confirmed in many resource-limited settings.

Prevention

Exposure control. Prevention of leptospirosis requires targeted strategies based on exposure risks, supported by epidemiological studies, integrated surveillance systems and improving diagnostic capacity. Mapping host-specific variability and the prevalence of *Leptospira* serogroups and strains are important for identifying the predominant animal reservoir(s) and infecting species in a region[151]. Such monitoring will enable the use of correct serogroup panels for the MAT and targeted vaccine development. Specific prevention and control measures include avoiding direct contact with shedding animals and contaminated environments, isolating infected animals, appropriate use of PPE, and vaccination of shedding animals with vaccines that protect against the regionally prevalent serogroups or species. Rodent control, proper waste management and wastewater drainage further reduce contamination risks[152]. Aside from animal vaccination, there is a lack of published epidemiological studies (experimental or observational) examining control strategies. Consequently, most guidelines (such as those published by the WHO)[153] rely on expert opinion and emphasize avoiding exposure and transmission or reducing contamination.

Preventive measures for leptospirosis should ideally be implemented at governmental (national or regional), organizational (private or public) and individual (includes households) levels. Nationally, governments can establish integrated pathogen surveillance systems in humans, animals and the environment, with mandatory human and animal case reporting, as well as public awareness campaigns. Depending on the epidemiological situation (subsidised) animal vaccination programmes might be useful for managing outbreaks and reducing transmission[154–156]. Regionally, local authorities and councils can address the risks by establishing clear communication on infection risks during floods or recreational events, allocating budgets for rodent control and improving urban infrastructure, such as providing safe drinking water, maintaining sanitation standards and waste management. At the level of organizations and enterprises with an increased risk of leptospirosis exposure, such as abattoirs or livestock farms, correct PPE use, vaccination policies for animals and staff awareness can reduce transmission[43]. Agricultural enterprises can fence off water sources to prevent contamination and isolate infected animals during outbreaks. At an individual level, people living in endemic areas should be cautious of water contamination (for example, following floods), wear PPE during high-risk activities, vaccinate pets, and manage rodent populations through traps and waste removal. Travellers to high-risk regions should seek the latest guidance on disease risks and how to avoid exposure[28].

Chemoprophylaxis. Chemoprophylaxis using subtherapeutic doses of antibiotics is an alternative preventative strategy for those at high risk of exposure, although high-certainty evidence for its efficacy is lacking[157]. A 2024 Cochrane review[157] of antibiotic therapy for leptospirosis prophylaxis identified five trials[158–162]. Methodological differences in these trials resulted in low certainty in the evidence and an indeterminate conclusion regarding the benefit of antibiotic prophylaxis (with doxycycline or azithromycin) on all-cause mortality or leptospirosis infection or the risk of adverse events. Only one trial showed a statistically significant benefit in preventing laboratory-confirmed infection[162] (Table 1). A meta-analysis[163] of two pre-exposure prophylaxis trials published in 2009 also showed no pooled beneficial effect of antibiotic prophylaxis in preventing deaths or laboratory-confirmed infections. However, given the small sample sizes and high risks of bias in these studies, the role of prophylaxis cannot be excluded with high certainty.

Vaccines. Effective vaccines to prevent leptospirosis in animals, adapted to local *Leptospira* strains, are available, and these might indirectly reduce human disease burden by preventing pathogen shedding[43]. Unfortunately, there is currently no safe and effective universal vaccine to prevent leptospirosis in humans[14]. Most human vaccine candidates so far have failed to progress to clinical trials owing to low efficacy and failure to demonstrate long-lasting, cross-protective immunity. For example, killed whole-cell suspensions have been trialled in Japan[164], Cuba[165] and France[166], and in China, a purified outer envelope vaccine has been developed[167]. Ultimately, however, the utility of vaccines against *Leptospira* has been limited due to poor long-term protection and lack of cross-protection against multiple serovars[168]. Regarding the latter, a single-dose vaccination with a live attenuated mutant (lacking the flagellar protein *fcpA*) induced cross-protective immunity against multiple serovars including *L. interrogans* in hamsters and mice to a greater extent than that observed with heat-killed vaccines[169]. The immune response was probably induced by transient bacteraemia after vaccination[169].

Two emerging areas of interest in leptospirosis vaccinology are DNA vaccines and subunit vaccines. DNA vaccines aim to induce both cellular and humoral immune responses by introducing a gene for an immunogenic pathogen antigen (such as the antigenic OMP of pathogenic *Leptospira*) into a host for in vivo antigen expression[170]. Progress has been made on several crucial steps towards a leptospirosis DNA vaccine[171], including identifying immunogenic and conserved pathogenic serovar-specific OMP genes[172–174] as antigens, as well as experiments to optimize plasmids for delivery of immunogenic genes to human cells[175–177] and to determine the optimal prime boosting strategy if multiple rounds of vaccinations are needed[176,178,179]. By contrast, subunit vaccine development focuses on identifying pathogen epitopes that can evoke an effective, long-lasting and cross-reactive immune response to multiple pathogenic serovars. Some *Leptospira* proteins (such as LigA, LigB, LipL41, LipL21, Loa22 and their fusion antigens (chimeric antigens that have epitopes of two or more naturally occurring antigens designed by immunoinformatics) are generating interest as potential vaccine candidates as they are conserved and specific to pathogenic serovars[110,180,181]. Selecting an appropriate adjuvant can further improve the efficacy and immunogenicity of a subunit vaccine[182,183]. For example, LigA coupled with the adjuvants AS04 or Montanide ISA720VG induces a stronger and longer-lasting antibody

Table 1 | Randomized controlled trials of antibiotic prophylaxis for human leptospirosis

Participants	Diagnosis method	Antibiotic	Comparator	Outcomes	Comments	Ref.
Paddy field workers in three towns of northern Iran (18–65 years of age)	New infections diagnosed by anti-*Leptospira* IgM ELISA using paired sera in symptomatic individuals (screening ELISA performed for all participants at baseline, week 6 and week 12)	Azithromycin (*n*=68) Doxycycline (*n*=71)	Placebo (*n*=61)	No difference in preventing new symptomatic and confirmed infections across any of the three groups	Double blinded but underpowered study	158
Community members with high risk of exposure due to flooding in Sao Paulo, Brazil (>18 years of age)	New infections diagnosed with anti-*Leptospira* IgM as measured by enzyme immunoassay	Doxycycline within 48 h of a flood (*n*=40)	Placebo (*n*=42)	No difference in preventing new symptomatic and confirmed infections	Double blinded but underpowered study	159
Farmers in Central Sri Lanka (20–80 years of age)	New infections diagnosed in symptomatic patients by the MAT using paired sera	Penicillin (*n*=319)	Placebo (*n*=167)	Inconclusive results due to high attrition rate and low event rates (only three new infections confirmed, all in placebo group)	High attrition rate and low compliance with medication	160
Community members of a single village in the Andaman Islands, Union Territory of India (>10 years of age)	New infections diagnosed with the MAT and culture	Doxycycline (*n*=513)	Placebo (*n*=512)	No difference in infection rates between the two groups, but reduction in symptomatic illness and deaths in the doxycycline group	Adequately powered double blinded study	161
US military personnel completing jungle training in Panama	New infections diagnosed with the MAT and culture	Doxycycline (*n*=469)	Placebo (*n*=471)	Reduction in new infections in the doxycycline group	Double blinded study. No sample size calculation provided	162

Trials were identified from a Cochrane systematic review that included trials published up to 2023 (ref. 157). The original papers were reviewed for verification and information extraction. The Primer authors then performed a systematic search in PubMed and Scopus (using the search terms 'Leptospirosis' AND 'Random' AND 'Control', without language restrictions) for studies published since then (1 January 2023 to 6 December 2024), and could not identify any new randomized controlled trials of antibiotic prophylaxis for leptospirosis. ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M; MAT, microscopic agglutination test.

and cytotoxic T cell responses in an animal model compared with alum as an adjuvant[183]. Advances in *Leptospira* genome sequencing, as well as transcriptome and proteome profiling, have also enabled reverse vaccinology approaches to identify potential candidates for vaccine development[184,185]. Overall, these advances are likely to accelerate the search for a suitable vaccine candidate in the next decade.

Management

Antibiotics

Antibiotics are recommended for treatment in early disease, as leptospires remain sensitive to many antibiotics including penicillins, cephalosporins, macrolides, tetracyclines and fluoroquinolones[15,111,153]. Late disease with organ dysfunction is often still treated with antibiotics, although the benefits are unclear. Such patients should be managed on a high-dependency unit or an intensive care unit with supportive treatment. Antibiotic sensitivity tests are not usually performed because the results take too long to be of any practical use and resistance is uncommon[186].

Mild cases can be managed in an outpatient setting with oral antibiotics such as doxycycline or azithromycin. In severe cases with the patient admitted to hospital, intravenous benzyl penicillin, doxycycline or third-generation cephalosporins such as ceftriaxone or cefotaxime have been recommended[153,187,188]. In children, the dose of antibiotics should be calculated according to their body mass, and in pregnancy, doxycycline is not recommended due to potential adverse effects on the developing fetus, and instead penicillin, cephalosporins and azithromycin may be used[189].

Whether antimicrobials are effective for leptospirosis is unclear. A 2024 systematic review of antimicrobial treatments identified nine eligible randomized controlled trials[190] (Table 2). The antibiotics assessed included penicillin, doxycycline, azithromycin, cefotaxime, ceftriaxone and chloramphenicol. The overall certainty of evidence was very low and the review concluded that there was a lack of definitive rigorous data from these trials to support the use of antibiotics for treating leptospirosis. The purported anti-inflammatory effects of ceftriaxone and doxycycline might also have a role in defervescence through immune modulation in addition to their microbicidal effect, although further study is needed[191,192].

The Jarisch–Herxheimer reaction (JHR), a clinical phenomenon that can occur after antibiotic administration has been reported after treatment with antibiotics for leptospirosis[193–195], probably due to acute inflammation triggered by LPS of dead *Leptospira*. The reaction is characterized by fever, rigors and hypotension and is treated by supportive management. In a study of 262 patients with leptospirosis, 21% developed a JHR; risk factors that were associated with JHR included infection with the *L. interrogans* serogroup Australis, and antibiotic administration within 3 days from the onset of symptoms[195]. Clinicians should be aware of JHR occurrence and how to manage it when treating leptospirosis.

Management of organ dysfunction

Organ dysfunction in severe disease is managed according to current guidelines with multidisciplinary input, as for any critically ill patient[196]. Acute kidney injury typically requires haemodialysis. Fluid balance must be carefully monitored with frequent electrolyte and blood gas

Table 2 | Randomized controlled trials of antibiotic treatment for human leptospirosis

Participants	Diagnosis	Antibiotics	Comparator	Outcomes	Comments	Ref.
Patients with leptospirosis from a single centre in Brazil (>15 years of age)	Direct microscopy, MAT or culture	Intravenous benzyl penicillin 4 hourly for 7 days (*n*=125)	No antibiotics (*n*=128)	No difference in deaths or duration of hospitalization	Open-label trial with a high risk of performance and detection bias	221
A single-centre study of patients with leptospirosis and renal failure in Brazil (18–55 years of age)	IgM class-specific antibodies in titres ≥1:400	Crystalline penicillin 6 million units per day for 8 days (*n*=16)	No antibiotics (*n*=19)	No difference in dialysis treatment indication or mortality	Small sample size; not clear how participants were randomized	222
Patients with icteric leptospirosis from a single centre in Barbados (39–40 years of age)	Direct microscopy, ELISA, MAT or culture	Intravenous benzyl penicillin 6 hourly for 5 days (*n*=38)	No antibiotics (*n*=41)	No difference in deaths, time to defervescence, time for liver function tests to return to baseline. Time to clearance of *Leptospira* in urine was faster in the treated group	Almost all patients were icteric and thus had more severe disease; not clear if blinding was performed	223
A multicentre study of British troops admitted to military hospitals with leptospirosis in Malaya (18–35 years)	Agglutination or culture	Penicillin 600,000 units every 6 h for 5 days (*n*=21)	No antibiotics (*n*=31)	No difference between the duration of illness in the three groups	Small sample size; not clear how participants were randomized	224
A single-centre study of symptomatic US military personnel returning from Panama	MAT or culture	Oral doxycycline twice daily, for 7 days (*n*=14)	Placebo (*n*=15)	Faster defervescence in the treated group	Small sample size; not clear if blinding was performed; no reported mortality	225
A single-centre study in Thailand recruiting symptomatic patients with leptospirosis (>16 years of age)	IgM rapid test and MAT	Intravenous ceftriaxone daily for 7 days (*n*=87)	Intravenous benzyl penicillin 6 hourly for 7 days (some patients also received gentamycin until diagnosis was confirmed) (*n*=86)	No difference in time to defervescence, duration of organ dysfunction or deaths	Open-label trial with a high risk of performance and detection bias	226
A multicentre study in Thailand recruiting symptomatic patients with leptospirosis (>14 years of age)	MAT	Oral azithromycin daily for 3 days (*n*=151)	Oral doxycycline twice daily for 7 days (*n*=145)	No difference in time to defervescence	Open-label trial with a high risk of performance and detection bias; high attrition rate; no reported mortality	227
A multicentre study in Thailand recruiting symptomatic adults with severe leptospirosis (33–35 years of age)	MAT, indirect immunofluorescence antibody test, microcapsule agglutination test or culture	Intravenous cefotaxime 6 hourly, changed to oral amoxicillin once afebrile and continued up to day 7 of treatment (*n*=88)	Intravenous benzyl penicillin 6 hourly, until afebrile and then converted to oral amoxicillin and continued up to day 7 of treatment (*n*=87). Intravenous doxycycline loading dose followed by twice daily dosing until afebrile and changed to oral doxycycline once afebrile and continued up to day 7 of treatment (*n*=81)	No difference in time to defervescence, duration of organ dysfunction or mortality rate	Open-label trial with a high risk of performance and detection bias	188
A single-centre study in the Philippines recruiting symptomatic patients with leptospirosis (>16 years of age)	MAT or culture	Intravenous benzyl penicillin 6 hourly for 7 days (*n*=23)	Placebo (*n*=19)	Shorter hospital stays and faster urine clearance of *Leptospira* in the penicillin-treated group	Small sample size, but the study was double blinded	228

One trial by Russell et al.[229] was not included in this table as the antibiotic tested (oxytetracycline) is no longer recommended for leptospirosis. Trials were identified from a Cochrane systematic review that searched the literature up to 2023 (ref. 157). The original papers were reviewed for verification and information extraction. Authors of this Primer then performed a systematic search in PubMed and Scopus (using the search terms 'Leptospirosis' AND 'Random' AND 'Control', without language restrictions) for studies published since then (1 January 2023 to 6 December 2024), and could not identify any new randomized clinical trials on antibiotic prophylaxis for leptospirosis. ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M; MAT, microscopic agglutination test.

monitoring, with hyperkalaemia aggressively managed. No specific dialysis modality is preferentially recommended, although continuous renal replacement is preferred in patients who are haemodynamically unstable. In resource-limited settings, obtaining early and optimal dialysis can be challenging. No data exist on the benefits of peritoneal dialysis, although there are no contraindications to its use. Myocarditis or pulmonary complications (such as acute respiratory distress syndrome and haemorrhage) must be managed in the intensive care unit. Patients with early signs of organ involvement (such as icterus, reduced urine output and rising serum creatinine) in rural centres with limited intensive care options must be transferred to a higher-level centre, as delayed transfers with advanced organ failure compromise patient safety.

The effectiveness of corticosteroids in the management of leptospirosis remains unclear. A 2015 systematic review[197] of high-dose corticosteroids for the treatment of severe leptospirosis identified five studies, including one randomized controlled trial, with none showing benefit except among patients with pulmonary involvement when corticosteroids were administered early.

N-Acetyl-cysteine has also been proposed as an agent to prevent or limit the severity of acute kidney injury in leptospirosis, based on its use in the treatment and prevention in contrast-induced nephropathy. However, the only available randomized controlled trial failed to show a benefit of *N*-acetyl-cysteine on renal function or duration of hospital stay in patients with severe leptospirosis infection[198].

Other treatment modalities such as plasma exchange, plasmapheresis and extracorporeal membrane oxygenation have been used by clinicians to treat severe leptosporosis, particularly when accompanied with severe pulmonary haemorrhage syndrome[199,200]. However, these treatment approaches are yet to be tested in a randomized controlled trial to show evidence of benefit[201].

Quality of life

Leptospirosis is often underdiagnosed, and it is difficult to estimate what proportion of undifferentiated febrile illnesses is due to leptospirosis, although it is likely to be higher in low- and middle-income countries than in high-income countries. Studies from Tanzania and Egypt show that up to a third of undifferentiated acute febrile illness is due to leptospirosis[202–204]. Severe leptospirosis accounts for 5–15% of diagnosed leptospirosis cases[110]. The annual global burden of leptospirosis is estimated to be 2.9 million disability-adjusted life years (DALYs) (uncertainty interval of 1.25–4.54 million)[205]. The economic burden of human infections is exacerbated by the impact in animals, as leptospirosis causes substantial yet often unquantified economic losses in livestock, primarily through reproductive failure and reduced milk production[206]. Moreover, clinical cases in dogs are associated with high veterinary costs. Most of the direct human disease burden is due to mortality, with years of life lost from mortality estimated at 2.80 million (ref. 205). Leptospirosis during pregnancy can have adverse effects on both mother and the child, though evidence from confirmed infections is limited. A systematic review found 35 instances of leptospirosis during pregnancy and a live birth was recorded in only 15 (43%) pregnancies[189]. No studies so far have examined the effect of social weighting based on age for the calculation of DALYs. However, as the disease primarily affects active and working men in low-income and middle-income countries, the economic impact is suspected to be huge, with an added indirect impact on families and communities, which is difficult to quantify.

Few cost-of-illness studies have been performed for human leptospirosis, with very little primary data[207–209]. Cost calculation based on estimated DALYs and per capita gross domestic product indicated that the global annual cost resulting from loss of productivity caused by leptospirosis was 29.3 billion Geary–Khamis dollars (a hypothetical currency unit that has the same purchasing power parity as the US$)[210]. Lower-middle-income countries were most severely affected, accounting for nearly half the total. The Southeast Asian region and Western Pacific regions had the highest economic burden, and the top five countries affected were China, India, Indonesia, Sri Lanka and the USA in terms of productivity cost.

Long-term sequelae of leptospirosis

Some patients remain unwell after the acute phase of leptospirosis. In a study from the Netherlands, fatigue, myalgia, malaise, headache, hair loss, mild jaundice and physical weakness leading to inability to work were reported at 1-month clinical follow-up in approximately 30–46% of patients who had recovered from confirmed leptospirosis[211]. These longer-term symptoms were more common in older patients than young patients. Thus, acute leptospirosis might also lead to a post-infection fatigue syndrome similar to that seen after some viral or bacterial infections[212], although more evidence is needed.

A proportion of patients who survive severe leptospirosis go on to develop chronic kidney disease (CKD)[213]. In a multicentre cohort study in Thailand, patients who recovered from severe leptospirosis had a significantly higher risk of developing CKD compared with those who had non-severe leptospirosis (adjusted odds ratio 3.22 (95% CI 1.04–9.96))[214]. Leptospirosis might also be one of the causative factors for CKD of undetermined cause (CDKu), a condition prevalent in Central America and Sri Lanka. A case–control study in a CKDu-endemic region of Sri Lanka showed higher seropositivity for leptospirosis among individuals with acute interstitial nephritis of unknown aetiology, compared with age-, gender- and occupation-matched controls in both CDKu-endemic and non-endemic areas[215]. A systematic review published in 2019, primarily based on two studies from Taiwan and Nicaragua, showed that in the general population, the estimated glomerular filtration rate was lower in *Leptospira* sero-positive individuals than in sero-negative people, after controlling for 17 confounding factors[216]. Two-year follow-up in a subset of these patients showed that the estimated glomerular filtration rate was lower among those whose anti-*Leptospira* antibody titres remained over 1:400. Progressive renal fibrosis seems to be the pathological mechanism by which CKD develops after leptospirosis infection, in combination with an imbalance of TGFβ and hepatocyte growth factor expression[217]. Although *Leptospira* colonize renal tubules in other mammals, especially rodents, evidence for renal colonization in humans is limited[104].

Outlook

More than a century after its identification, major gaps in knowledge still exist regarding the pathophysiology of severe leptospirosis. Mortality rates for leptospirosis remain higher compared with those of other similarly prevalent tropical infections, such as dengue infection. Despite clear evidence of its impact, leptospirosis has yet to be added to list of neglected tropical diseases (NTDs) by leading public health authorities, such as the WHO, even though it meets the established criteria for a NTD[218–220]. This exclusion contributes to a lack of funding and political awareness, resulting in minimal surveillance and control efforts across human, animal and environmental sectors. Furthermore, there is insufficient investment in research, vaccine and RDT development, exacerbating diagnostic challenges, under-reporting, and

limited awareness among health-care professionals and vulnerable communities.

Climate change and urbanization are likely to change the demographics of the disease, as the zoonotic reservoirs are ubiquitous. There is a paucity of research on leptospirosis, especially on developing point-of-care diagnostics, vaccines and specific treatments for severe disease. Physicians working in resource-poor settings are often faced with notable challenges when attempting to treat previously healthy adults who are affected by this deadly disease. In such settings, high-dependency and intensive care facilities, renal replacement therapy, and even inotropes and vasopressors, are often unavailable, and basic standards of organ support are unachievable. Plagued by limited resources for funding and poor visibility, the neglect of this potentially fatal disease will impede the achievement of several United Nations Sustainable Development Goals.

Knowledge gaps

Several critical knowledge gaps should be prioritized in future research. Global disease burden and mortality rates for leptospirosis were last estimated in 2015 (ref. 10). An update in these numbers is greatly needed, especially in view of changes in climate, demography, population size, urbanization and rise in recreational travel across countries since 2015 and especially after the COVID pandemic. Research into the eco-epidemiology of leptospirosis, including the study of prevalences in different human habitats stratified by population density (urban and rural), geography (islands and estuaries) and socioeconomic status, is another priority. Future projected changes in disease prevalence in these locations should also be addressed, particularly in the face of a globally changing climate.

Regarding pathophysiology, a comprehensive understanding is lacking about the minimum infectious dose in humans, pathogenesis of severe disease, duration of immunological memory or its cross-protection against other serovars after an infection, and the influence of host and pathogen genetic mutations on disease severity. In terms of diagnostics, accurate and cheaper point-of-care diagnostics, in particular those identifying pathogen antigens, are needed. When testing their comparative efficacy, Bayesian latent class modelling should be used when possible rather than assuming MAT to be the gold standard[13]. Genomic diagnosis is more compatible with the new taxonomical classification of *Leptospira*, but alternative ways of detecting the presence of pathogen genome must be explored as sequencing is too costly for resource-limited settings.

Other important areas of research that should be prioritized include the development of a universal, effective and safe vaccine for human leptospirosis. DNA vaccines might be a way forwards, but further research is needed to achieve better immunogenicity for current experimental candidates. Also needed are high-quality randomized controlled trials, including those comparing one antimicrobial agent against the other, to improve the evidence base for the effectiveness of antibiotics in treating leptospirosis. Finally, targeted immunotherapies are needed to prevent or treat the cytokine storm or immunoparesis in severe leptospirosis.

Prioritization of leptospirosis research within the above themes, and recognition of it as a NTD by the WHO to develop a globally effective action plan based on a comprehensive One Health approach to minimize transmission, would help to reduce leptospirosis-related morbidity and mortality in the next decade and beyond.

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Informed consent
The authors affirm that human research participants provided informed consent for publication of the images in Fig. 4.

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