



Australian Government

Department of Health
and Aged Care



Australian
Centre for
Disease
Control

2025 • Volume 49

Communicable Diseases Intelligence

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Communicable Diseases Intelligence

Communicable Diseases Intelligence (CDI) is a peer-reviewed scientific journal published by the Health Security & Emergency Management Division, Department of Health and Aged Care.

The journal aims to disseminate information on the epidemiology, surveillance, prevention and control of communicable diseases of relevance to Australia.

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ISSN: 2209-6051 Online

This journal is indexed by Index Medicus and Medline.

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Abstract

Leptospirosis is a zoonosis caused by exposure to *Leptospira* excreted into the environment by rodents or other mammals. A notification of a case of leptospirosis in an adult male with no history of travel or exposure to livestock or rodents triggered an environmental health investigation of his workplace, a local golf course. We hypothesised that a water splash in the eye from a creek running through the golf course, which occurred after a period of heavy rainfall, had led to *Leptospira* exposure, likely on the basis of contamination of the creek water by rodent urine. Testing of environmental water samples detected pathogenic *Leptospira* DNA in ten of eleven samples, although cultures were negative. However, we had difficulty interpreting this finding as we found *Leptospira* DNA in ten of 14 environmental samples in inner and eastern Sydney remote from the workplace, and these were not associated with notified human cases. When we reviewed the 53 human leptospirosis cases notified over the twenty-year period 2003–2022 in residents of metropolitan Sydney, of the 49 cases with *Leptospira* exposure information, 46 had recognised sources of exposure: travel overseas (27) or to tropical northern Australia (5); rural exposure often to livestock and/or rodents (12); work in an abattoir (1); and involvement in a raspberry farm outbreak (1). Only three, including the case described, acquired infection in suburban Sydney. Acquisition of human leptospirosis is a rare event in suburban Sydney; true cases without a travel or occupational exposure history may be under-recognised by clinicians. However, with increasing biodiversity loss and where climate change results in heavier rainfall and more frequent floods, it is likely that human leptospirosis will become more common in urban as well as endemic settings.

Keywords: human leptospirosis; *Leptospira*; urban acquisition; environmental health; environmental microbiology; zoonoses

Background

Human leptospirosis is a zoonosis of global occurrence caused by infection with spirochaetes of the *Leptospira* genus. *Leptospira* are widely distributed in the natural environment, particularly in water and moist soil. Whilst the genetic characterisation of *Leptospira* is an emerging field, the use of whole genome sequencing (WGS) has led to identification of at least 64 species which are divided

into three groups: saprophytic, pathogenic (for humans and other mammals) and intermediate.¹ Pathogenic strains are classified into over 250 serovars which vary in their global distribution and host reservoir. The World Health Organization (WHO) Collaborating Centre for Reference and Research on Leptospirosis, a division of Queensland Health, identifies 22 serovars of Australian relevance.²

Human infection follows penetration of pathogenic *Leptospira* through abraded skin or the mucosa. It may present as a relatively mild influenza-like illness, with conjunctival suffusion or pneumonia and less often with life-threatening meningoencephalitis, acute respiratory distress syndrome or multiorgan failure.³ It generally follows exposure to either soil or water that has been contaminated with the urine of reservoir mammals, particularly rats and mice, which generally have chronic, subclinical renal infection, or by direct, often occupational, exposure to infected domestic or feral animals. Presumptive laboratory diagnosis of leptospirosis includes detection in blood of *Leptospira* species immunoglobulin M (IgM) by enzyme immunoassay or of *Leptospira* DNA by polymerase chain reaction (PCR) testing. Definitive diagnosis of infection and implication of a specific serovar currently rely on use of the microscopic agglutination test (MAT).

Globally, it is estimated that approximately one million cases and 60,000 deaths occur each year, with the highest incidence in tropical countries among men of working age.⁴ In Australia, where leptospirosis is a nationally notifiable disease, the highest incidence occurs in the wet tropical regions, primarily in Queensland and the Northern Territory. Over the past twenty years, the annual incidence in other states and territories has been less than or equal to 1 case per 100,000 per year, whilst in Queensland it has been in the range 0.8–4/100,000/year and in the Northern Territory 0.4–6/100,000/year.⁵ In these two jurisdictions, an increase in case numbers has been associated with flooding with or without occupational exposure to animals,^{6–8} whilst recent rainfall appears to have been a factor in a large outbreak among raspberry farm workers in northern New South Wales.⁹ Other important risk factors are work in agricultural industries such as cattle farming, meat processing and banana growing, and feral pig hunters; many cases follow overseas travel to tropical areas, particularly when it involves recreational water contact.¹⁰

Leptospirosis is thus strongly associated with residence in or travel to tropical areas or with employment in rural industries. Therefore, in cities within high-income countries, leptospirosis is rare and may not be considered in the diagnosis of an illness which is often non-specific.³

In June 2022, we were notified of a case of leptospirosis in a resident of southeastern Sydney who had not travelled outside of Sydney in the exposure period, and we believe that this was the first urban-acquired

case in this part of Sydney in over twenty years. This prompted us to undertake an environmental health investigation of the local source of his infection and to review sources of cases in residents of metropolitan Sydney in the past two decades.

Methods

Case report

Public Health Unit staff interviewed the case following receipt of the notification and on several subsequent occasions. We supplemented this information through discussion with the case's hospital medical team and review of hospital electronic records, including pathology and imaging reports. The case provided written consent to the publication of a clinical case report.

Environmental health investigation

Leptospira ecology is an emerging field, and the understanding of the classification and pathogenicity of these organisms is still evolving. Strains referred to as 'pathogenic' in relation to animals or humans may contaminate the environment through the deposition of urine from infected mammals, particularly rodents.

In the absence of a history of rural or overseas travel, we speculated that the case's place of work might be the source of his exposure. Environmental health officers from the Public Health Unit undertook a site visit to assess the potential for exposure to surface water and to rodents or other mammals. This investigation was supplemented by microbiological sampling and testing in partnership with the Elizabeth Macarthur Agricultural Institute (EMAI) of the New South Wales Department of Primary Industries.

Microbiological methods

Samples of water and water sediment were tested for *Leptospira* DNA at EMAI using a published real-time quantitative PCR (qPCR) assay developed for clinical diagnosis of human leptospirosis that targets an 87 base pair (bp) sequence of the *rrs* (16S) gene of pathogenic *Leptospira* species,¹¹ as recently applied.¹² DNeasy PowerSoil Pro DNA extraction kits (Qiagen Inc., Toronto, ON, Canada) and Instagene DNA extraction kits (BioRad, Hercules, CA, USA) were used to extract DNA from 0.25 g of water sediment and from 50 mL of pelleted water respectively, according to manufacturers' instructions.

An in-house-designed *Leptospira* spp. plasmid standard, the 87 bp *rrs* target from a positive field sample received at EMAI and cloned into a pCR2.1 TOPO™ vector (ThermoFisher, Waltham, MA, USA), was created and used for all qPCR runs as a positive control and to form a standard curve to quantify results. Eight replicates of six dilutions of the *Leptospira* plasmid DNA, at 20, 2, 0.2, 0.02, 0.002 and 0.0002 fg/μL, were used to calculate a limit of detection (LOD) using GraphPad Prism software Version 4.02 (San Diego, CA, USA) with a nonlinear regression model and a sigmoidal dose-response. The 50% LOD, utilised to determine the cut-off between a positive and negative result, was determined to be 0.026 fg/μL.

Water samples were additionally set up for *Leptospira* culture in Ellinghausen McCullough, Johnson and Harris (EMJH) media (Edwards, Narellan, NSW, Australia) and incubated aerobically at 30 °C for eight weeks. Samples were examined at high-power under dark-field using a light microscope for *Leptospira*-like organisms and motility; fresh subcultures were set up weekly by transfer of 600–900 μL to fresh EMJH media.

Review of cases residing in metropolitan Sydney 2003–2022

For the review of cases notified in residents of metropolitan Sydney, a standard report was obtained from the NSW Health Notifiable Conditions Information Management System (NCIMS) which comprised a line listing of cases of leptospirosis in residents from the Local Health Districts (LHD) of South Eastern Sydney, Sydney, Northern Sydney, South Western Sydney, Western Sydney and Nepean Blue Mountains, with calculated onset date between 1 January 2003 and 31 December 2022. The report was exported to a spreadsheet containing the following fields: unique NCIMS number; birth date; gender; address; calculated onset date; classification (confirmed or probable); owning jurisdiction (i.e. LHD); and serovar. Each NCIMS record was reviewed by one author [MJF], (a) to determine likely exposure (this field was added to the spreadsheet), and (b) to add serovar information where this was missing and where MAT results which indicated a serovar were available in NCIMS.

Publication of this aggregate New South Wales leptospirosis notification data was approved by the Executive Director, Health Protection NSW.

Results

Case report

In June 2022, the Public Health Unit was notified of a *Leptospira* species IgM antibody-positive serum sample from a previously healthy man in the 60–69 years age group, who had been admitted to hospital in May with a five-day history of fever, rigors, headache, myalgia, lethargy and dry cough. Examination was significant for mild conjunctival suffusion and bi-basal inspiratory crackles. On admission, he was noted to have mildly abnormal liver function tests (bilirubin 22 μmol/L [reference range 0–20]; alkaline phosphatase (ALP) 337 units/L (U/L) [30–110]; gamma-glutamyl transferase (GGT) 204 U/L [5–50]; aspartate transaminase (AST) 93 U/L [reference range < 36]; alanine transaminase (ALT) 73 U/L [< 51]); and stage 1 acute kidney injury (creatinine 111 μmol/L, estimated glomerular filtration rate (eGFR) 61 mL/min). Abdominal ultrasound showed no evidence of cholecystitis or choledocholithiasis. A computed tomography (CT) scan of the chest showed bilateral small pleural effusions. He was commenced on empirical oral doxycycline and completed a ten-day course.

Serological testing during hospital admission in May was significant for detection of an equivocal *Leptospira* species IgM and equivocal *Mycoplasma pneumoniae* IgM. Other microbiological investigations, including viral serology (cytomegalovirus, Epstein-Barr virus, hepatitis B virus, hepatitis C virus, human immunodeficiency virus) and nucleic acid testing for respiratory pathogens (viral, atypical), were non-contributory. Serology performed on convalescent samples collected three weeks later confirmed the diagnosis of leptospirosis, with a positive *Leptospira* IgM (equivocal immunoglobulin G), and a MAT titre of 1:1600 against serovar Arborea. Subsequent nucleic acid testing on stored serum (collected during hospital admission) detected *Leptospira* DNA, with notification at the end of June.

When we received the *Leptospira* IgM notification, the case was interviewed by a public health nurse using a standard questionnaire. In the exposure period, defined as 2–30 days before onset, he had no travel history to rural or tropical Australia or outside of the country. There were cats in the household, but he stated that they were well and denied any close contact with them.

He reported working as a groundskeeper for a local golf course, and that he routinely wore protective footwear and clothing. He had not sustained recent cuts or abrasions and had no direct animal contact at work, although he stated that the golf club had in the past implemented some rat control measures. He also reported that the creek flowing through the course had flooded in the period prior to his illness as a result of heavy rainfall. His regular duties included using a brush cutter to trim vegetation around the creek, and he stated that during this activity some water had splashed into his eyes and mouth.

Environmental investigation

In October 2022, approximately five months after the putative exposure had occurred, an environmental health inspection was carried out at the golf course where the case worked. The investigation was delayed as it took time to identify a laboratory able to test environmental samples for *Leptospira* spp. It was noted that there was a food outlet on the course with an open food waste storage area adjacent to it; this and nearby vegetation cover were thought to be attractive to vermin, although rats were not observed during the daytime inspection. Due to recent rainfall, standing water was observed in this area. During the inspection, the manager of the golf course was advised that staff should wear eye protection to reduce the chance of the eyes being splashed with potentially contaminated water.

At this visit, samples for *Leptospira* DNA testing were collected at multiple sites across the golf course. Pathogenic *Leptospira* DNA was detected in ten of eleven water and sediment samples (threshold cycle [Ct] range 33.6–37.5; concentration range 0.04–0.50 fg/ μ L).

Purified *Leptospira* DNA from the three water sediment samples with the lowest Ct values (33.6, 34.0 and 34.2) were sent for sequencing (AGRF, Westmead, NSW, Australia). Unfortunately, the sequencing data was low quality and *Leptospira* speciation was not possible. All six water samples were *Leptospira* culture negative.

In light of the detection of *Leptospira* DNA in most golf course samples, in January 2023 we decided to collect samples from the local freshwater system both downstream and upstream of the golf course, and from locations in inner Sydney unassociated with the local water system. In this investigation, *Leptospira* DNA was detected in four of eight water and sediment samples from the local system (Ct range 30.9–36.2; concentration range 0.076–2.46 fg/ μ L) and in all six water and sediment samples from unassociated inner Sydney sites (Ct range 33.0–37.8; concentration range 0.026–0.60 fg/ μ L).

Review of cases residing in metropolitan Sydney 2003–2022

The New South Wales Health NCIMS database recorded 53 cases of leptospirosis in residents of metropolitan Sydney with onset from 1 January 2003 to 31 December 2022, predominantly among men (87%; n = 46) and ranging in age from 17 to 70 years.

Likely exposures were assessed to be: travel overseas, 27 (51%), or to tropical northern Australia, 5 (9%); travel to or farming in New South Wales rural regions, often with mention of rodent or farm animal contact, 6 (11%); farm or surface water exposure on Sydney's rural fringe, 6 (11%); and other occupational exposure, 2 (4%), namely an abattoir worker and a berry picker involved in the raspberry farm outbreak.⁹ Only three cases (6%), including our case, appeared to have been acquired within suburban Sydney, including one with known rat exposure. The remaining four cases (7%) were lost to follow up.

A likely infecting serovar based on MAT results was available for 43 cases, summarised in Table 1.

Table 1: Likely *Leptospira* serovar of cases notified in residents of metropolitan Sydney, 2003–2022, by whether acquired in Australia or overseas^a

Serovar	Australian acquired	Overseas acquired
Arborea	9	0
Australis	2	6
Canicola	2	5
Copenhageni	0	5
Hardjo	3	4
Zanoni	0	3
Grippotyphosa	1	2
Tarassovi	0	1
Unknown	8	1
Total	25	27

a One case was diagnosed postmortem, and neither place of acquisition nor serovar was available.

Discussion

We initiated an environmental health investigation because we believed that urban acquisition of leptospirosis has been a rare event in Sydney residents and that the possibility of workplace acquisition was worth investigating in view of the implications for wider exposure of employees and members of the public. Our investigation confirmed the plausibility of workplace exposure to pathogenic *Leptospira*. The Arborea serovar which caused our case's infection is found worldwide in rats and mice, and in Australia its range extends from North Queensland to Victoria.² Wildlife experts provided advice that rats were very likely to roam from the nearby natural bushland to the area where the case worked and to urinate in and around the creek where the case had a water exposure; additionally, the putative exposure occurred after a period of heavy rain. In April and May 2022, the period immediately preceding this case's illness, Sydney experienced above average rainfall, with slightly warmer minimum temperatures whilst maximum temperatures were in the usual range.¹³

Pathogenic *Leptospira* have been found in both laboratory and field conditions to survive for weeks or months in water and soil,¹⁴ including in soil from sites which cases had visited 6–17 weeks earlier.¹⁵ Further, pathogenic *Leptospira* were found to not just survive but multiply in waterlogged soil, suggesting a mechanism for increases in cases after heavy rain or flooding.¹⁶

Reports of human leptospirosis cases from large cities in high-income countries, including Baltimore,¹⁷ Marseille,¹⁸ Tokyo,^{19–20} and New York,²¹ found that urban-acquired cases were uncommon. Where environmental sources were sought, cases could often be attributed to contact with rats or their urine.^{17–19}

Some of these studies also reported microbiological findings in rats as part of the case exposure investigation, with pathogenic *Leptospira* detected in 17–90% of rats.^{17–19} A recent study of rats trapped within the boundaries of the City of Sydney, prompted by an increase in canine cases, reported detection of pathogenic *Leptospira* DNA in the kidneys of 9 of 111 rats (8%). MAT serology from a small subset of rats was negative so that it was not possible to ascertain serovar.¹²

Our review, of cases in residents of metropolitan Sydney over the twenty-year period 2003–2022, found that only three of 53 cases (6%) appear to have been acquired in an urban setting: a person who kept chickens and reported rat exposure; a person who had bushwalked; and the current case who appears to have been exposed to *Leptospira* serovar Arborea in his outdoors workplace. Whilst the low number of cases considered to have arisen from urban exposure may be an accurate reflection of incidence, it has been proposed that, due to a low index of suspicion and the non-specific clinical presentation of most cases, in relatively affluent urban settings without obvious occupational or travel risk factors, cases are likely to be under-diagnosed.^{3,22,23}

It is noteworthy that in our twenty-year review of Sydney cases, out of those with a serovar recorded, Arborea infection was diagnosed in nine of the 17 Australian acquired cases (53%) and all nine infections were acquired in New South Wales. This contrasted with the absence of Arborea and a much wider range of other serovars implicated in cases acquired overseas.

The high prevalence of *Leptospira* DNA in environmental samples collected during our investigation, using an assay which was designed to detect pathogenic serovars, is hard to explain in the absence of more widespread human disease. It is possible that the assay which was developed for clinical diagnosis is not suited to testing of environmental samples which may contain a variety of pathogenic *Leptospira*. Recent genomic studies of tropical soils identified novel *Leptospira* spp. which fell into the pathogenic category but were of lower virulence.²⁴ We await the development of strain-specific nucleic acid detection methods which can be applied to soil and water samples, as these will help to better define the potential for exposure and hence disease risks.

In Australia,⁷⁻⁸ the Pacific,²⁵ and globally,²⁶ outbreaks have often been associated with flood events, which are becoming more frequent due to climate change. As a result of population growth, advancing urbanisation, and a decline in biodiversity all increasing human exposure to pathogenic *Leptospira* in the environment,²⁷ it is likely that human leptospirosis will become more common in coming years in urban as well as endemic settings.

Acknowledgments

We wish to acknowledge the role of staff of New South Wales Public Health Units in following up leptospirosis notifications. These notifications may only represent a fraction of the actual number of persons with the disease in the community. The number of notifications reflects that health care seeking behaviour and testing practices may vary across New South Wales. Consequently, these data are likely an underestimation of the true incidence of infections in New South Wales. We also thank Dr Vicky Sheppard for her support and advice, and Rebecca Limbach for her assistance in reviewing the literature.

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